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THE

ORIGIN OF LIFE:

BEING AN ACCOUNT OF EXPERIMENTS WITH CERTAIN SUPERHEATED SALINE SOLUTIONS IN HERMETICALLY SEALED VESSELS

BY

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FOREWORD

In this volume a memoir is reproduced on "The Origin of Life Question" that was submitted early last October to the Royal Society, and which, as I was informed after an interval of a few weeks, was "not considered suitable for acceptance by the Society."

I desire, therefore, to make a few observations in the first place in reference to the subject of the paper; and, secondly, in regard to the action of the Society, and the probable causes that have led to such action.

Concerning the main question—that of the Origin of Life on this Earth—men of science, or at least a majority of them, no longer appeal to the intervention of any non-natural or miraculous cause. As believers in the Doctrine of Evolution they are content to suppose that at some time after the fiery heat of the crust of our globe had sufficiently cooled to permit of the deposition of water upon its surface, there must have been a further continuance of the physico-chemical processes that had gradually led to the evolution of all the inorganic elements and their compounds from the primal stuff of which the parent-nebula of our solar system was composed.

These further physico-chemical processes, whose real nature is unknown, even though their result was the production of what we now know as "living matter," with all its marvellous qualities and potentialities, could only be regarded as the forging of other and more complicated links in the chain of synthetic processes by which it had been preceded.

This must be said, although it is true that with the birth of living matter there is, of course, the origin of what we know as

"Life." But what is "Life"? From the scientific point of view life is no entity—it is only the summation and aggregated result of all the properties of living matter. This sum-total of properties and potentialities must vary for every particular living thing, in accordance with its complicacy of structure and function, together with its past personal and ancestral history.

What has been said indicates sufficiently what is meant by the "natural origin of Life," as opposed to its origin by some supernatural or miraculous agency.

The question then clearly arises whether such a life-giving process (which I term Archebiosis) occurred only once, or at all events only in the very early days of the Earth's history, or whether it is one that has ever been taking place since the period when it first began.

The majority of scientific men seem to favour the former point of view. They appear to think that conditions may have existed in those comparatively early days of the Earth's history more favourable to this life-evolving process than those which have since obtained; although, of course, their notions as to the actual nature of these primeval conditions must be almost as dim and theoretical as any conceptions to which they may incline concerning the actual conditions needful for the occurrence of Archebiosis.

Starting points, however, having once been given by means of these early evolutionary processes, all the forms of life that have ever appeared on our globe are now commonly supposed by men of science to have been gradually evolved, by processes of "natural selection" and otherwise, from such primordial types.

Others consider that the life-originating processes may have been many times repeated over many parts of the Earth, though not in recent times.[1] While a third comparatively small section,

[1] The consequences of this "Polyphyletic" doctrine in reference to the course of, and time needed for, organic Evolution, together with the interpretations to be attached to fossil remains from the point of view of genetic relationship or otherwise, are most important, and would naturally often differ much from those favoured by the "Monophyletic" doctrine, to which Darwin, Huxley, and so many others, have seemed to incline.

to which the writer belongs, incline to the belief that life-evolving processes are now and have ever been going on, in suitable sites, since the times when they first commenced.

In adopting this latter view, we rely upon the known Uniformity of Natural Phenomena, and believe that just as all ordinary physical and chemical processes that have once occurred in any part of the universe are apt to recur, so we have a right to believe in the strong probability that the physico-chemical processes which originally led to the birth of living matter would similarly and constantly tend to be reproduced.

Another very strong point in favour of our view, and one that I have during many years urged, is the fact that the lowest forms of life still exist all over the surface of the earth, as might be expected if they have been and are constantly surging up from new-born units of living matter. But the doctrine of evolution being true, it seems impossible to suppose that such simple and at the same time highly mutable living things could have gone on reproducing their like, with little or no change, through all the untold ages of the world's history. Does not the evolution theory of itself tend to negative such a supposition, implying, as it does, that living things, owing to the nature of the matter of which they are constituted and their intrinsic tendency to undergo change, have, in fact, been continually though slowly changing, as evidenced by the past and present extraordinary diversity of the representatives of the Vegetal and the Animal Kingdoms?

But the weighing in the balance of these mere relative probabilities will never suffice to settle the question whether living things are or are not still coming into existence by means of so-called "spontaneous generation." Nature has to be questioned by the institution of proper experiments.

Even so long as 150 years ago simple experiments of a kind akin to those described in the present work began to be made in this country by Turberville Needham (who very shortly thereafter was elected a Fellow of the Royal Society), and in Italy by the Abbé Spallanzani, with a view to see whether any distinct evidence

could be obtained of a *de novo* origin of living things in certain infusions of organic matter.

After a considerable interval, that is in 1837, similar investigations were set on foot in Germany by Schultze and Schwann, and later (1851) in Italy by Professor Mantegazza. The question was taken up again in Germany in 1854 by Schroeder and Dusch, while about seven years later a celebrated controversy was commenced in France, and carried on for some time between Pasteur, Pouchet, Joly, Musset, Trécul, Fremy, and others, in reference to the same question—Pasteur denying and the others adducing evidence in favour of the occurrence of "spontaneous generation."

A similar experimental enquiry was initiated by Professor Jeffries Wyman in America in 1861, and was renewed in Italy in 1868 by Professors Cantoni, Balsami, and Maggi ; and each of these last four workers obtained results that were distinctly opposed to those of Pasteur, and in favour of the *de novo* origin of micro-organisms.

In 1870 the controversy was revived in this country by the publication of my first experiments, together with a celebrated Presidential Address from Professor Huxley, in which, though not having worked at the subject himself, he strongly supported the views of Pasteur. Other experiments were published by me from time to time during the next seven years, and the results of some of them were confirmed by Professor Burdon Saunderson (much to his surprise), as well as by Professor Huitzinga, of Groningen ; though I was rather savagely attacked by Professor Tyndall, who himself caused much bewilderment by the extremely contradictory results of different series of his experiments. Towards the close of this period a controversy was started between Pasteur and myself that led to the appointment of a commission of the French Academy of Sciences, the result of which, owing to various causes, was abortive.

An account of these experiments from 1870–1877 is to be found in my work entitled *The Evolution of Life.*

After the latter date (1877) I ceased to work at the subject (or

rather to publish anything) for a very long period, not because I was convinced that my opponents were right and I was wrong, but because all my energies were needed in other directions, and such controversies as had been carried on were proving harmful to my career as a young physician. When, however, I had served for thirty years at University College and its attached hospital, I resigned my professorship and my physiciancy (1898) in order that the freedom from teaching and from hospital work might enable me to devote more time and energy to problems which I had apparently altogether relinquished.

I knew full well the thankless and arduous work that lay before me in order to procure even the semblance of a fair hearing. For during all these intervening years the important science of Bacteriology had steadily grown up, and its fundamental principles were generally thought to be incompatible with my views and experimental results. I was, in fact, supposed to have been beaten out of the field, and my silence during these years perhaps lent some support to the notion.

During the first six years of the renewed investigations they were devoted to a cognate subject—namely, Heterogenesis—at which I had previously worked in 1870–1872. Heterogenesis is the name applied to processes by which living things arise from the matter of pre-existing organisms belonging to a totally different species. Or, in other words, as I have defined it, it is "the production from the substance of organisms or their germs of alien forms of life." These investigations resulted in the publication, towards the end of 1903, of a large fully-illustrated work, entitled *Studies in Heterogenesis*, in which many facts were recorded and views expressed at variance with commonly received biological doctrines.

One set of examples may be cited. If the reader were to refer to the index of this volume he would see after the word "Amœbæ" that there are references to descriptions of about five-and-twenty different modes in which I have seen such organisms forming from the substance of other vegetal or animal matrices. Thus I have

seen Amœbae taking origin from the protoplasm of some of the cells of Moss radicles, from the substance of encysted Euglenæ, from the eggs of different kinds of Rotifers, from Vaucheria resting-spores, from encysted Ciliates, and from the Bacterial scum on hay infusions and on emulsions of egg and water. In these latter situations myriads of minute specimens may usually be found. Yet among all the multitudes of Amœbæ that I have seen in these and other situations, multiplication even by simple fission has only rarely been witnessed, and the formation of brood-spores never, either with or without previous "conjugation."

The occurrence of this latter process, or of any other mode of spore formation among the naked Amœbæ, is stated, in all the best works on "The Protozoa," to be extremely rare. Calkins, for instance (p. 96), could refer only to two doubtful cases in which such a process had been seen; and the extreme rarity of its occurrence is similarly admitted by Cash, Marcus Hartog, and Ray Lankester, who are the principal authorities on this subject.

In explanation of what I have seen and described, these authorities would never admit the occurrence of Heterogenesis; they would at once appeal to their facile supposition that some "infection" had occurred in each and every case. But infection by what? By their own confession any spores or germs of Amœbæ that could possibly infect this or that matrix are so rare as to be practically unknown. Surely the existence and action of such bodies ought not to be postulated gratuitously and independently of all evidence. Moreover, in many of the cases *the whole substance* of the resting-spore, the Euglena or the Rotifer's egg, *may be seen to have undergone simultaneous segmentation into Amœbæ*.

What has just been said concerning the heterogenetic origin of Amœbæ might be repeated in very similar terms in regard to some Ciliated Infusoria, except that such an origin for them has been observed more rarely. The instances are, however, quite as impossible of any real explanation otherwise than by heterogenesis. Germs of Ciliates are similarly unknown which could be supposed to have "infected" the great encysted Amœbæ or the great

Rotifer's eggs, which at times segment, also without any remainders, into specimens of this or that particular kind of Ciliate, as I have seen and have illustrated by photographs in the work above mentioned.

But the notion of the possibility of Heterogenesis is generally repudiated, and is scarcely ever likely to find favour except with those who believe that living matter is still constantly coming into existence *de novo*. My facts and views in this direction received, therefore, little or no recognition of a favourable kind, and as the work in which they are embodied is expensive, I produced, in 1905, a smaller book, entitled *The Nature and Origin of Living Matter*, in which, among other subjects, an account was included of some of the most remarkable heterogenetic changes that I had observed.

Towards the end of this same year I began again to take up experimental work, in an endeavour to find further proof of the continued occurrence of a *de novo* origin of living matter—the process which Huxley termed Abiogenesis, but which I had previously named Archebiosis.

This time my attempts to procure evidence of the occurrence of the process were made, not with infusions or solutions of organic matter, such as had been almost solely employed in previous years by others as well as by myself, but with mere saline solutions such as would alone have existed on the surface of the Earth when life-evolving processes were first initiated.

Some striking results were obtained in these experiments in 1906 with solutions that in each case contained small quantities of colloidal silica and a few other ingredients, and which had been exposed to very high temperatures.

Details concerning these experiments were brought before the Medical and Chirurgical Society of London, and have been published in Vol. XC. of its *Transactions;* but as up to last year no one seemed to have repeated these experiments, I took up the problem again, with the definite intention of slightly varying the composition of the solutions, and trying to make the results of the experiments more uniformly successful.

In this I succeeded to a very remarkable extent, so that the last series of experiments made during this year were of such a decisive nature, and so comparatively uniform in their results, notwithstanding the very high preliminary temperatures to which the vessels and their solutions had been exposed, that they seemed to me adequate to finally solve the much-debated question as to the Origin of Life, upon which so many important biological as well as philosophical issues depend.

Naturally, therefore, and notwithstanding previous experiences, I desired to submit my results to our premier scientific society—the Royal Society of London.[1] This was accordingly done early last October. The paper seemed to me to contain so much evidence of painstaking work that, when coupled with the nature and importance of the results which it chronicled, I certainly never anticipated receiving, as I did a few weeks later, an official letter saying that the paper "had been under consideration, and that it was not considered suitable for acceptance by the Society."

This was the bald statement made, and, as usual, not a word of explanation! Its rejection could not be owing to the nature of the subject dealt with, since two long papers by Professor Tyndall had been accepted and published by the Society several years ago, as well as others by Sir William Roberts.

One can only conclude, therefore, that now, as at that time, when another paper of mine was similarly declined and was deposited in the archives of the Society, it is the kind of conclusion that has to be drawn from the experiments which is obnoxious to some influential members of this Society "for the promotion of Natural Knowledge."

The two referees to whom the paper was handed, it may be safely assumed, whatever their general qualifications, had never

[1] Four of my communications to the Royal Society from 1876 onwards had not been accepted; and of a fifth, a long memoir on some very important instances of Heterogenesis, an illustrated abstract only was allowed to appear, with a modified title which now stands as follows: "On the Occurrence of certain Ciliated Infusoria within the Eggs of a Rotifer, considered from the point of view of Heterogenesis." (See *Proceedings of Royal Society*, B, Vol. 76, 1905, p. 385.)

themselves done any similar work, but they did not in any way communicate with me; and, although the paper was subsequently submitted to a joint committee, it is perfectly clear that the members round that table, as a committee, could obtain no adequate knowledge of the contents of the paper. They had to be influenced in the main by the report of the two referees. The Council, sitting as a committee of papers, would be in much the same condition, and, unless some very strong influence was interposed, would, in the ordinary course of things, ratify the previous decision, depending upon the report of two referees by whom alone the paper would have been read—as it actually did in this case.

Some such process is doubtless necessary for the elimination of unfit contributions, but it is one which surely ought to be very jealously guarded in the case of a paper from a senior Fellow who was known to have devoted much time and labour to the subject of his investigation—and more especially when the conclusions arrived at point to some very distinct advance in Natural Knowledge. What is the object of the Society but to advance Natural Knowledge? And how can it expect to do this if it tries to stifle or ignore that which is adverse to generally accepted beliefs?[1]

The same kind of thing was done by the Society in the case of one of Joule's papers on "The Mechanical Equivalent of Heat," though his investigation soon became one of the most important contributions to the doctrine of "The Correlation of the Physical Forces," enunciated by Mr. Justice Grove in 1842.

So far as my own case is concerned, I have little doubt that, when I ventured to submit this paper to the Society, I did not adequately appreciate the amount of reluctance there would be to receive any new knowledge from one whose views were known to

[1] The action of the Society is all the more surprising seeing that on one of the first pages of every volume of the *Philosophical Transactions* the following statement is to be found in reference to the selection, by the Committee, of papers for publication therein: "And the grounds of their choice are, and will continue to be, the importance and singularity of the subjects, or the advantageous manner of treating them, without pretending to answer for the certainty of the facts, or propriety of the reasoning contained in the several papers, which must still rest on the credit or judgment of their respective authors."

be so very unorthodox—especially as the results of the experiments recorded would still probably be held to be absolutely at variance with the everyday procedures of bacteriologists. A distinguished physiologist indeed recently suggested to me as an *a priori* objection to my results that, if true, "the whole of modern bacteriology, with its isolation of different forms of bacteria in pure cultivation, must go by the board, since no method of sterilisation could be held to prevent the development of micro-organisms spontaneously differing from those which had been placed in any given cultivating medium by inoculation."

But this is a mistake, as I have elsewhere pointed out. The different results in the two cases are entirely due to difference of aim and procedure. The bacteriologist wishes to make doubly sure that the media he uses will no longer contain living micro-organisms or their germs. Therefore he superheats them to a degree sufficient to destroy what I may call the "germinality" of the fluids, while leaving such fluids still capable of nourishing the organisms introduced by inoculation. He heats them, therefore, two or three times to 100° C. or else once to 130° C., that is to a temperature far higher than would be needed to kill any organisms that could possibly be found in his tubes, and looking to the organic nature of his media, which would probably be sufficient to destroy their "germinality." My objects are, of course, quite different. What I try to do is, while heating the fluids and vessels sufficiently to kill all pre-existing living things, not unnecessarily to degrade or break up the colloidal compounds contained in the solutions (though such as exist in my saline solutions seem to resist much higher degrees of heat than those that are to be found in organic solutions); and then, after a time, to examine very carefully the *minute and unobtrusive deposits* that are to be found in the vessels. For in these high-temperature experiments there is never the production of general turbidity. The organisms are always comparatively scarce and to be found only at the bottom of the vessel.[1]

[1] In regard to the media of the bacteriologist there are two points which have perhaps hitherto been insufficiently considered in this relation:

Another important direction, however, in which my work appears to come into conflict with that of bacteriologists is in its application to public health doctrines. My views, while in no way adverse to doctrines of contagion, favour the notion that infectious or communicable diseases may, at times, and under special conditions, still arise *de novo* as they must certainly once have done, unless some special creative acts are postulated. But to such doctrines bacteriologists are, as a rule, strongly opposed. They are ultra-contagionists, and for the most part seem disposed to ignore the possibility of the *de novo* origin of such diseases.

There are, however, some notable exceptions, and I would especially call attention to an authoritative statement made by Lehmann and Neumann in their *Principles of Bacteriology* (Translation 1901, pp. 118–119), in which they say: "The division of bacteria into pathogenic and non-pathogenic, etc., as is still always done in text-books, has failed absolutely. We can understand and know the pathogenic varieties only if we study simultaneously the non-pathogenic, *from which the former have once originated and still always originate.*" They say also: "We certainly believe it belongs to the future to convert varieties of bacteria into others in a manner scarcely to be imagined to-day," and then proceed to give reasons for this belief. Here, then, apart even from *de novo* origination of the bacteria, there is the admission by celebrated bacteriologists of doctrines like mine which are commonly deemed altogether unorthodox. Still, as Lehmann and Neumann say, there are many indications of an approaching change of view.[1]

(*a*) That suitable uninoculated media which have been kept for some time, and had not been too much superheated, have never had their *minute and unobtrusive deposits* scrutinised; and (*b*) that the changes set up by inoculation would not be interfered with by any such processes as have been referred to above. The inoculation changes would begin to occur at once, and would probably render abortive any other much slower processes that might otherwise have occurred.

[1] As further evidence of the truth of this statement the reader may be referred to an article by M. C. Potter entitled "Bacteriological Research in Phytopathology" (*Science Progress*, October, 1910, p. 209), in which he says: "With a knowledge of the fact that nutrition may so alter a facultative saprophyte that it becomes a virulent parasite, while through other

But for biologists also, as I have already pointed out when speaking of Heterogenesis, my views are similarly unpopular, and to botanists especially the results recorded in this memoir will seem well nigh incredible. Biologists have been led to believe, as Herbert Spencer and others have suggested, that anterior to the appearance of living things on the Earth there would probably first have been a very slow elaboration of some proteid compounds before the actual production of protoplasm in some amorphous condition like the hypothetical Bathybius of Huxley, followed in the course of time by the evolution of actual Amœbæ.

How, then, could those holding such views look, in the first place, except with extreme incredulity, at experimental results which show that not only Bacteria of well-known types, but that Torulæ of most varied kinds with the potentiality of straightway growing into simple forms of well-known Moulds, are some of the common, everyday exemplars of new-born living matter?[1] The difficulty would doubtless be great, and the evidence would require to be irresistible before such persons could renounce all their preconceptions and accept facts which at first sight seem incredible.

But the evidence is irresistible, as may be realised when it becomes obvious that, on the one hand we have to do only with hypotheses and preconceptions entirely devoid of any direct evidence, and on the other the results of simple experiments, over and over again repeated, showing that Torulæ and Moulds, as well as Bacteria, may be obtained practically at will from hermetically sealed tubes that have been heated to 125°, to 130°, to 140°, and even to 145° C. (293 F.); while, as every text-book says, Torulæ

nutritional changes its virulence may be entirely lost; and, further, that the same influence operates in rendering the host more or less susceptible, we have the key to one of the most important determining factors in the epidemic diseases of plants." Such are the views that skilled experts are now beginning to enunciate. They are in marked contrast to popular doctrines concerning communicable diseases, in accordance with which a Cambridge professor, when speaking of Tuberculosis and the very great importance of minimising its spread by means of contagion, has lately ventured to say (*The Times*, Sept. 20, 1910) "the disease can no more be developed than can turnips spring up where no seed has been sown."

[1] As to this point, see what is said in Section X., p. 65.

would invariably be killed by momentary exposure in fluids to a heat of 70° C., or even less.

Yet the organisms taken from these super-heated tubes are not dead organisms, but living Torulæ and Bacteria, which must have been born therein, and which soon, under favourable conditions, begin rapidly to grow and multiply. Here, then, surely, we have the actual Origin of Life demonstrated as far as such a thing is possible.

Happily, it can no longer be said, as was formerly the case, in reference to my early experiments, that the organisms found were dead, and had been there all the time. This was the criticism actually started by Huxley, and subsequently taken up by others, because of his strong disbelief at that time that any Bacteria could survive in boiled infusions, and his equal disbelief that they were capable of originating in such fluids (see *Quart. Journ. of Microscop. Science*, Oct., 1870, pp. 359, 362). He suggested, in fact, that the organisms I had found in my experimental vessels were only organisms previously contained in the fluid, but killed by the heat to which they had been subjected.

Nor is it possible for any reasonable person to suppose that, in taking the samples from the just-opened tubes by means of sterilised pipettes, organisms could have been derived from the air during the passage of the sample (within the pipette) to the microscope slip. Looking to what has been immediately found on microscopical examination, and which is shown by the photographs (mostly taken within half-an-hour), any such view cannot be deemed worthy of serious consideration.

In the face of the results of these experiments is it or is it not important that the experiments should be repeated by others? The Royal Society would appear to think that any confirmation of Natural Knowledge in this direction is not desirable.

That may be so; but I take a different view, and sincerely hope that others may repeat these experiments and seek to obtain similar results. All that will be needed are some tubes of soft soda glass, similar to those that I have employed, a strong can

containing a quart of colza oil, a good thermometer, some distilled water, and three or four simple chemicals, details as to which will be found in the paper. No incubator is necessary. The sealed and superheated tubes may be left at rest inside a closed south window for one, two, three or more months before opening them and most carefully examining their contents. The last point is all-important, for without the exercise of much care it will be easy enough for an unbeliever or even a hasty observer to fail to discover any organisms.

FURTHER EXPERIMENTS ON THE ORIGIN OF LIFE QUESTION WITH CERTAIN SUPERHEATED SALINE SOLUTIONS IN HERMETICALLY SEALED VESSELS

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I.—INTRODUCTION

AUGUST WEISMANN, in his work on *The Evolution Theory*, after referring to the observations of Pasteur concerning the prevalence of germs in the air, and the fact that his experiments tended to show that their exclusion from boiled organic

infusions caused such infusions to remain barren, makes the following remarks[1] :—

> Strangely enough, these and similar experiments were at the time regarded as conclusive proof against the existence of spontaneous generation, though it is obvious enough that the first living being on the earth cannot have sprung from hay, or from any other organic substance, since that would presuppose what we are attempting to explain. After the fiery earth had so far cooled that its outermost layer had hardened to a firm crust, and after water had condensed to a liquid form, there could at first only have been inorganic substances in existence. In order to prove spontaneous generation, therefore, it would be necessary to try to find out from what mingling of inorganic combinations organisms could arise; to prove that spontaneous generation could never have been possible is out of the question.....It would be impossible to prove by experiment that spontaneous generation could *never* have taken place; because each negative experiment would only prove that life does not arise *under the conditions of the experiment.* But this by no means excludes the possibility that it might arise under other conditions.

Haeckel is no less emphatic in his repudiation of the too wide and positive nature of the conclusions drawn by Pasteur and others from the results of their experiments with organic infusions. In his work *The Wonders of Life* he says[2] :—

> These experiments prove nothing whatever beyond the fact that new organisms are not formed in certain infusions of organic matter—under definite artificial conditions. They do not even touch the important and pressing question, which alone interests us : "How did the earliest organic inhabitants of our earth, the primitive organisms, arise from inorganic compounds?"

He, therefore, like Weismann, while taking a more sober view as to the nature of the conclusions that Pasteur and other observers were entitled to draw from experiments

[1] *Loc. cit.*, translation, 1904, vol. ii., p. 366.
[2] Translation, 1904, p. 367.

attended by negative results, also points to the importance of dealing with inorganic materials, if the question of the possible present-day origin of living matter is sought to be established.

If Pasteur, Tyndall, and others, drew conclusions from their experiments wider than were warranted by their premises, they, as well as all other workers at this problem, intermediate between Spallanzani and themselves, at all events firmly stamped with their authority the nature of the experiments by which alone, if ever, the question of the present-day *de novo* origin of living units could be settled. Certain materials were to be enclosed in hermetically-sealed vessels, and both were then to be exposed to what existing knowledge regarded as lethal temperatures. Secure in their belief that this was the means by which the problem was to be solved, many of them, upon the basis of their negative results, came to the conclusion not only that there was no evidence that living matter could arise independently—they went further, and more or less loudly proclaimed that spontaneous generation was a vain or idle fancy.

What, then, is to be said if, dealing with solutions containing only inorganic materials, contained in hermetically-sealed vessels, and exposing them to degrees of heat that have been shown to be fatal to every known living thing, simple organisms nevertheless appear again and again within the tubes—always of approximately similar kinds, and of kinds well-known to be killed at temperatures only a little more than half as high as that to which the experimental vessels and their fluids had been originally exposed?

The mode of experimentation cannot now be repudiated or deemed inadequate. It is the same as that formerly employed, and from whose negative results the belief has been widely spread through the scientific world that

spontaneous generation is a myth. If, then, in other experiments, of a similar kind except that inorganic materials have been employed, and that the initial destructive temperature has been much higher and such as all positive knowledge entitles us to believe would make the experimental vessels as void of living things as was the earth when its surface first began to cool below the boiling-point of water—if, I say, in such cases simple organisms can be shown to appear almost at will in the experimental vessels, we should obtain the best possible warrant for the conclusion that "spontaneous generation" is no myth, and that simple living units of well-known kinds can now be evolved, even within experimental vessels, as other living things must originally have been evolved on the cooling surface of the earth.

If a genesis of living matter occurred in some one place in far-remote ages, and if such a process can be shown still to occur, it would be only natural to conclude that the same chemico-physical processes have, in all probability, been operative in innumerable regions over the surface of the earth, not only in primeval but in all succeeding ages up to the present day.

I have long held such a belief upon the basis of my own experiments, of the type made by Pasteur, Tyndall, and others, with organic infusions, but in which positive results were obtained not otherwise explicable in accordance with existing knowledge.[1] This belief has, during the last four years, been notably strengthened by the results of experiments, many times repeated, made with inorganic materials, and now to be further referred to.

I was not deterred from making attempts in this direction by the discouraging remarks of Weismann in the passage

[1] See a memoir "On the Conditions Favouring Fermentation, and the Appearance of Bacilli, Micrococci, and Torulæ in Previously Boiled Fluids," *Linnean Society's Journal* (Zoology), vol. xiv., 1877.

following that which I have already quoted, where we find him saying (p. 367) :—

> Up till now, all attempts to discover these conditions have been futile; and I do not believe that they will ever be successful—not because the conditions must be so peculiar in nature that we cannot reproduce them, but, above all, because we should not be able to perceive the results of a successful experiment.

This latter remarkable statement he then endeavours to support by reason of the assumed incompatibility of the *de novo* origin of a simple living unit, such as a Bacterium or a Torula, with his own well-known theories.

Altogether apart from theories, however, when Weismann says, in reference to such researches, " We should not be able to perceive the results of a successful experiment," we must agree with him, of course, that we could not perceive the initial combinations and all the stages that lie beyond the range of our aided vision ; but when the previously invisible, newly-evolved germs grow into minute but definite organisms, we surely could perceive the results of our successful experiments. Similar reasoning would just as much entitle Weismann to say that we never could perceive the birth of a crystal from its mother liquor because its initial combinations would lie altogether beyond the range of our vision, however aided. Nevertheless, we know full well that crystals appear and grow in this or that fluid into prismatic, hexagonal, and other forms, just as we may assume that the new-born units of living matter, as they come into the region of the visible, may take on the shapes of Micrococci, Bacilli, and Torulæ. If these are the forms that appear in the experimental vessels, we should necessarily have to conclude that new-born units of living matter speedily take definite but highly variable forms, just like new-born units of crystalline matter.

Nor is Haeckel more encouraging to any would-be investi-

gator of this problem (*loc. cit.*, p. 367). He dwells mainly upon two difficulties: first, that at the time "when organic life first appeared on the cooled surface of the earth—at the beginning of the Laurentian age—the conditions of existence were totally different from what they are now," and that their nature is very imperfectly known to us; and, secondly, after referring to the extreme complicacy of the molecules entering into the composition of living matter, he says: "As long as we are ignorant of this complex structure of albumen, it is useless to attempt to produce it artificially."

But surely neither of these reasons need deter any experimenter from making an attempt to deal with inorganic materials in the same kind of way that others had dealt with organic infusions. The question is, in each case, what may be possible by natural processes, under the restrictions necessitated by the conditions of the experiment. The actual steps of the process in the genesis of living matter are problems that lie altogether outside such inquiries. These far more difficult laboratory problems connected with the building up of complex organic compounds must be left to be dealt with by the combined researches of chemists and physicists. We may, however, profitably inquire whether solutions can be devised which, after thorough sterilisation and continued protection from outside contamination, will, as a result of natural tendencies, and after weeks or months of exposure to particular conditions, lead to the appearance therein of living units. If so, many old and widely accepted theories would have to give place to altogether new views having most important bearings upon many departments of natural knowledge.

II.—CONCERNING EXPERIMENTS MADE IN 1906

THE present research is a continuation of one a report of which was presented to the Royal Medical and Chirurgical Society in December, 1906, in a communication entitled "On the *de novo* Origin of Bacteria, Bacilli, Vibriones, Micrococci, Torulæ, and Moulds in certain previously superheated Saline Solutions, within Hermetically Sealed Vessels," which is to be found in Vol. XC. of the *Transactions* of that Society.

The experiments to which reference was there made were of this nature. Two simple saline solutions were employed, one of which was mostly of a yellow colour, and contained to each ounce of distilled water only a few drops of a dilute solution of sodium silicate, together with about three times as many drops of liquor ferri pernitratis; while the other was colourless, and contained in each ounce of distilled water a few drops each of a dilute solution of sodium silicate and dilute phosphoric acid, together with a few grains of ammonic phosphate.

Portions of these solutions were introduced into sterilised tubes of soft glass about one inch in diameter and three inches long, but drawn out beyond to a tapering extremity.[1] When about half filled with one or other of the experimental solutions, the necks of the tubes were carefully sealed with the aid of a Bunsen's burner, and the tubes were subsequently immersed in a calcium chloride bath, which was raised to temperatures ranging from 115° to 130° C. for from ten to twenty minutes.

These sealed and heated tubes were then exposed, to diffuse daylight, or else were placed in an incubator maintained at a temperature of 30°–33° C. In the former case they were kept on a tray just inside a window facing the East,

[1] These were always made for me by Messrs. Müller, Orme, & Co. of 148 High Holborn, W.C.

which remained open day and night, because I had previously found that growth and multiplication of Bacteria and Torulæ occurred in these saline solutions under the influence of diffuse daylight more readily than in similar solutions kept in an incubator and at temperatures very much higher.[1]

In each of the solutions a considerable amount of deposit was produced by the heat, and when, as "controls," some of these tubes were opened within a day or two after the heating, and portions of this deposit were examined with the microscope, no organisms of any kind could be found amongst it; though, after the tubes had been exposed for some weeks or months to the influence of diffuse daylight or to heat in the incubator, organisms of different kinds were then very often to be found in abundance on the deposited silica or ferric silicate, and there only. They were never found in the supernatant fluid, which invariably remained clear. The organisms, moreover, were always motionless; so that, as I then said, "If organisms are not there at first, after the process of heating, and if, after an interval, they are there in abundance and are invariably stationary, clearly they must have developed in the sites where they are found."

Details of many of the experiments were given, illustrated by photographs of some of the organisms found in tubes which had been heated to different temperatures from 115° to 130° C.;[2] and after citing what was known concerning the thermal death-point of the organisms found, as previously determined and admitted by bacteriologists, the experimental results were embodied in the following statement: "It comes to this, then, that all the organisms found in my experiments, with the exception of Bacilli, are such as would be killed at

[1] See *Knowledge and Scientific News*, Aug., 1905, p. 199.

[2] The number of experiments described with illustrative photographs will be found to be more numerous in my work, subsequently published, entitled *The Evolution of Life*, 1907.

100° C.; that these latter, so far as they could by any possibility be found within my tubes, should have been killed in one or two minutes at 115° C.; yet Bacilli, as well as Bacteria, Vibriones, Micrococci, Streptococci, Torulæ, and other Fungus-germs, dying under 100° C., have been taken in large numbers from tubes that had been heated to 115°–130° C. for ten to twenty minutes."

III.—NEW EXPERIMENTS INITIATED IN 1909

As I had seen no account of attempts by others to repeat these experiments, and as I was anxious to discover the best proportions in which to use the different ingredients of the two solutions—one of which, for convenience of reference, I shall speak of as *yellow*[1] and the other as *colourless*—I made last year, between June and August, 105 other experiments with hermetically sealed vessels heated from 100° to 135° C., in addition to very many other comparative and tentative trials in which the fluids were heated to 100° C. for ten minutes, and were not contained in hermetically sealed vessels.[2]

In many of these tentative experiments I inoculated solutions, having the ingredients in different proportions, with Bacteria which had been developed either in organic infusions or in solutions containing neutral ammonic tartrate and sodic phosphate, so as to try and find out which were the particular combinations in the experimental solutions most favourable for the growth and multiplication of micro-organisms, and, therefore, possibly also most favourable for their origination.

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[1] Though they are often after the heating process rather more red than yellow.

[2] Concerning such experiments see p. 31, *note*.

Such comparative trials were very necessary, because among the very numerous experiments made with these solutions in 1906 there were many negative as well as many positive results; and I was naturally anxious to discover the means by which the proportion of positive results might be notably increased.

Before speaking in detail concerning these experiments with hermetically-sealed vessels which were initiated last summer, it is necessary to call attention to a very important point concerning the ingredients used in the making of my solutions. Of these, ammonic phosphate, dilute phosphoric acid, and liquor ferri pernitratis are definite pharmaceutical products, which ought to be severally uniform in their composition whenever and wherever procured. It is not so, however, with sodium silicate, which is a variable commercial product.

I was formerly not as fully alive to this fact as I ought to have been, or I should have provided myself in 1906 with a good supply of this substance from the same stock as that with which my experiments were then made. As it was, I had to get a new supply last summer; and, having obtained it from the same house, at first supposed that I was dealing with just the same kind of ingredient as had been previously used. It proved, however, to be somewhat different in strength and composition; and the sequel has been that in these new experiments positive results were only obtained after periods three or four times as long as those which sufficed in the trials made in 1906.

I had found then that in spring and summer weather (and the latter happened to be specially warm and bright) the diffuse daylight experiments were much more successful than those made in the autumn and early winter of the same year, when both light and heat were much less. And as all the

new experimental vessels were this time to be exposed to diffuse daylight,[1] and half the summer was over before the first batch of tubes was exposed (on a protected balcony facing the East), I did not open any of them till six months had elapsed—that is, till last January. Then, and during the first half of February, I examined the contents of a large proportion of the twenty hermetically sealed tubes that had been heated only to 100° C., and was much surprised at finding no organisms in any of them.

That first led me to suspect some difference in the constitution of these solutions, and to make inquiries as to whether the sodium silicate used for the latter experiments was from the same stock as that which had been sold to me and used in 1906. I was then informed that the supply of last summer had come from a different stock; and on comparing it with a small quantity of the old preparation still in my possession, some distinct differences between them were ascertained. I was, therefore, induced to get supplies of sodium silicate from several other sources, and to make many comparative trials with solutions varying only in the one respect that the sodium silicate was from a different source. The results of these trials have served to show that solutions made from a supply obtained from Allen and Hanbury early in March last, and heated only to 100° C. for ten minutes, would give positive results in about the same time as the other solutions did that were made with sodium silicate obtained from Martindale in 1906; that is, in four to six weeks for the colourless, and three to four months for the yellow solutions.[2]

[1] Owing to my residence in the country, and having no means of making proper use of an incubator.

[2] This sample of sodium silicate has a specific gravity of 1.44, though it is always used by me diluted with an equal bulk of distilled water. A tin containing some of the silicate of this make has been reserved by Messrs. Allen and Hanbury at 6 Vere Street, Oxford Street, at my request, in case others may wish to obtain some of the same sample for a repetition of the experiments.

The results of all these comparative trials have led to some other important conclusions. I have found, for instance, that, whatever sample of sodium silicate has been used, organisms were ultimately to be discovered in the solutions. Some specimens, however, yield them much more quickly than others. I have also, as a result of examining solutions in which only a minimum amount of deposit was present, come to the conclusion that in the previous series of experiments the difficulty of finding the organisms has been needlessly great owing to much more sodium silicate than was necessary having been used in the composition of the solutions and the consequent throwing down of a large amount of deposit, from amid which the organisms had to be discovered, although they were very minute and at times not very numerous.

Then, again, as the different samples of sodium silicate vary in their degree of alkalinity, and as, for the obtaining of positive results, it is desirable to use solutions having a faintly acid or neutral reaction, the best course to pursue is for the experimenter to make solutions with two, three, or four drops of the dilute sodium silicate to the ounce of distilled water, the proportion of the other ingredients remaining always the same (that is, six grains of ammonium phosphate and six drops of dilute phosphoric acid for the colourless solution, and merely eight drops of the liquor ferri pernitratis for the yellow solution). He will then test for acidity or neutrality, boil each of the solutions for ten minutes, and, some time after they have cooled, compare their respective amounts of deposit, so as to see which is the best number of drops to use with the particular specimen of sodium silicate, in order to obtain a faintly acid or neutral solution with only a very small amount of deposit. In experiments of this kind we require, in the first instance, only to know whether living organisms are or are not to be found; we

do not require to find any large number of them, and if they are there they may be found much more easily in the small than in the large amount of deposit.[1]

In regard to this latter point—that is, the finding of organisms—when the deposit is considerable it is often a matter of great difficulty, requiring much time and patience. If they are very abundant, they may be found readily in the first sample of the sediment taken with a sterilised pipette from the just opened tube. But at other times, when present only in small numbers, two or three samples may have to be examined and one or two hours spent, with the light carefully adjusted, before any of the sparsely distributed organisms can be discovered.

Again, even the organisms most commonly met with, the Torulæ, are often extremely minute (see Plate 3, Fig. 14), and thus difficult to be found with low powers of the microscope. I have little doubt but that in the early months of 1910 I failed to find organisms in several of the tubes because I trusted to being able to detect them with too low a power. Up to April 30 I had been habitually searching for organisms with a one-inch objective and a No. 6 compensation eye-piece. But on that day I began to adopt the more laborious method of diligently searching through the whole sample of the deposit with a quarter-inch objective and the

[1] In these tentative trials it is best to use thoroughly sterilised two-ounce flasks, subsequently fitted with previously boiled india-rubber stoppers, having in each a small wedge-shaped portion cut out of its lower extremity, so that the stopper when loosely standing in the neck of the flask may allow of the passage of the steam, and yet when the ebullition has ceased may permit of the flask being securely closed. And in the preparation of these solutions, when small quantities only are often required, it is best to use a dropper. With that which I have always employed 40 drops equal 36 minims of the dilute sodium silicate, 38 minims of the dilute phosphoric acid, and 40 minims of the liq. ferri pernitratis. In preparing the colourless solution it is best to dissolve the ammonium phosphate first, then add the acid, and lastly the dilute sodium silicate.

same eye-piece; and the result has been that organisms have been found in every one of the remaining thirty-seven tubes whose contents were examined. In several cases the organisms were so minute that they would almost certainly have escaped observation had I been searching only with the low power. Still, in part this increased number of positive results has doubtless been due to the fact of the longer times that had elapsed after the heating process before the tubes were opened. Thus, out of the total number of 105 trials, in this series of experiments, with hermetically-sealed tubes heated to temperatures ranging from 100° C. to 135° C., there have been sixty-six positive results; while in eighty-five of these trials, in which the tubes had been heated from 120° C. to 135° C., there were no less than sixty positive results. The failures among the twenty tubes heated only to 100° C. actually numbered fourteen, and were so very numerous apparently because the sample of sodium silicate employed was not a favourable one, and solutions in which it was used would yield organisms only after long periods. The tubes making up these fourteen failures had been examined after seven to nine months, while the six tubes yielding positive results had been examined after nine to twelve months.[1] And similarly, among the eighty-five trials above referred to, the twenty-five negative results were with tubes which had been opened after seven to nine months, and the sixty positive results with those which had been opened after nine to twelve months, and whose contents had also been more thoroughly examined.

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[1] The exposure being largely to open-air winter temperatures and dull illumination.

IV.—THE VARYING EFFECTS OF HIGH TEMPERATURES UPON THE SOLUTIONS

THE varying effect of high temperatures is shown best in regard to the yellow solution. The changes produced in it have been found to differ in a remarkable way even when the solutions contain only very slightly varying amounts of sodium silicate to the ounce of distilled water.

Thus the tubes Nos. 80, 81, 82, and 92, 93, 94, were all heated together in the can to 125° C. for ten minutes; and the result was that in each of the first three tubes the solution was decomposed, all the iron silicate being thrown down in the form of a copious reddish yellow deposit, while the fluid above was perfectly clear and colourless. But in each of the other three the deposit was very scanty, and the fluid itself was reddish and opalescent. Yet the only difference was that the first three tubes were each charged with a solution containing six drops of the dilute sodium silicate, and the other three with a solution containing four drops of the same to the fluid ounce, the quantity of the iron solution being similar in each set—namely, eight drops to the ounce.

Then, again, Nos. 89, 90, 91, and 101, 102, 103, were likewise heated together in the can, but to a temperature of 135° C. for ten minutes; and every one of these solutions was completely decomposed. There was a copious reddish yellow deposit in each of the tubes, while the fluid above was as clear and colourless as distilled water. These two sets of tubes had been charged with some of the same solutions as those above referred to—containing respectively six and four drops of the dilute sodium silicate to the ounce.

After ten months the deposit in Nos. 80 and 101 was examined with the microscope, and it was found to resemble very minute fragments of pounded glass of a reddish yellow colour, among which no organisms of any kind could be

discovered; though the slight flocculent deposit in Nos. 92, 93, 94 contained Torulæ and Bacteria in abundance.

I had, however, previously heated six tubes, Nos. 37, 38, 39, and 43, 44, 45, to 135° C. for five minutes, which were all charged with some of the yellow solution, containing twelve drops of the dilute sodium silicate and six drops of the iron solution to the ounce of distilled water; and these particular solutions were not at all decomposed—that is, there was only a small amount of yellowish deposit in each, while the fluid itself remained of a pale yellow colour. Moreover, in five out of these six tubes organisms were found, when they were opened after nine to eleven months[1] (see Plate 5, Figs. 25 and 28).

At other times, when the yellow solution has been decomposed so as to leave only a clear, colourless fluid above the deposit, this latter, instead of being composed of mere minute, glass-like fragments, has been in the form of a copious reddish, gelatinous material, in which organisms have sometimes been found and sometimes not.

Again, there are distinct colour variations, resulting from the use of different proportions of sodium silicate when the quantity of the iron preparation remains the same in each case—that is, eight drops to the ounce. Thus, with two drops to the ounce, after boiling for ten minutes, the fluid with one sample had a pale port-wine colour, with scanty deposit; with four drops to the ounce the colour of the fluid was a gamboge yellow, and the deposit more abundant; while with six drops to the ounce the colour of the fluid, after the same amount of heating, was pale yellow and opalescent, and the deposit still more plentiful.

[1] Six tubes, charged with the colourless solution, containing twelve drops of the dilute sodium silicate to the ounce (Nos. 34, 35, 36, and 40, 41, 42), were also heated to 135° C. for five minutes; and in three of these tubes organisms were found, when opened after eight and a half or eleven months (see Plate 4, Fig. 24; and Plate 5, Figs. 26, 27).

As the strength of different samples of the sodium silicate varies so much, trials should be made with solutions containing one, two, three, and four drops to the ounce which have been boiled for ten minutes, in order to ascertain the number of drops of the dilute sodium silicate in use that will give this kind of pale port-wine colour to the solutions, together with only a very scanty precipitate; as I have generally found such solutions to be most productive.

No variations of the kind to which I have been referring are, of course, observable when dealing with the colourless solution. Here it is only a question of much or little deposit from the use of varying quantities of the dilute sodium silicate, together with variations in the acidity or alkalinity of the solutions, that can be observed. I may say, however, that with this solution the aim should also be to use only one to three drops of the dilute sodium silicate to the ounce, instead of eight or twelve drops, as I had been previously employing. This, as I have now realised, was a distinct mistake, since it, in the first place, enormously increased the difficulty of finding the organisms, when not very numerous, in the midst of the copious deposit produced; and, secondly, because, as I have since found, only very small quantities of silica are really necessary.

I have, in fact, now come to the conclusion that whatever specimen of sodium silicate is being employed for the preparation of the colourless solution, only such an amount should be used as, after boiling for ten minutes, will yield almost no deposit when the fluid has cooled. This minute amount of deposit will, in the course of a few days, undergo a very slight increase.

I have previously called attention to some of the effects of different temperatures on these colourless solutions, and to

differences produced when the tubes employed were of uviol rather than of ordinary soft soda glass.[1]

This colourless solution is, doubtless, completely decomposed at temperatures slightly above 145° C., although the fact of such decomposition is not, as in the case of the yellow solution, recognisable by mere inspection.

V.—CONCERNING THE EXPERIMENTAL CONDITIONS, THE NATURE OF THE ORGANISMS FOUND IN THE TUBES, THEIR CULTIVATION AND THEIR THERMAL DEATH POINT

Little requires to be said concerning the experimental conditions beyond what has already been stated.

After the process of heating, the tubes were cleaned, labelled, and exposed to diffuse daylight at the end of an open north balcony facing the east. There they remained all through the winter, and most of them for nine to twelve months, till, day by day, one was opened for examination of its contents.

In the month of April, however, a few of the tubes were brought indoors, and placed just inside a south window, where they were left for five or six weeks before they were opened. I was induced to take this step because I had found, rather to my surprise, that in some of the tentative experiments, where the flasks had been so exposed, the conditions had proved very favourable. They had certainly had the advantage of a much warmer temperature, and the influence of the sunlight through the thick glass of the window, as well as that of the experimental tube, if not helpful, seemed clearly not harmful. The Torulæ subsequently found in these tubes

[1] *The Evolution of Life*, 1907, pp. 266, 274.

were often unusually abundant and large (such as are shown in Plate 5, Fig. 26). Bacteria, too, have been often plentiful in these particular tubes.

In regard to the nature of the organisms taken from the tubes generally, an examination of Plates 1–5 will show that they have been Bacteria of different kinds, Torulæ, and Moulds; though other bodies have at times been met with, apart from obvious crystals and mere concretions, concerning whose nature there has been room for doubt.

The photographs from which the illustrations have been made were mostly taken at once—that is, as soon as the samples of deposit, abstracted with a carefully-sterilised pipette, had been mounted on a clean slide, and microscopical examination had revealed their presence. Only a selection from the very numerous photographs taken has, of course, been reproduced.

Details concerning each illustration will be found in the explanation of the Plates, but it will be useful here to call attention to some particulars.

It may be seen that the Torulæ vary very much, not only in size, but in character. Thus, in some they are minute and separate, as in Plate 3, Fig. 14, and Plate 4, Fig. 20 B; while in others they are quite large, and form continuous groups, as in Plate 3, Fig. 17. Frequently they look homogeneous and glistening, as in Fig. 1; while at other times they have distinctly granular contents and thick walls, and show, perhaps, vacuoles or else commencing hyphæ (Plate 1, Fig. 5).

The Bacteria, too, have been of different kinds, Bacilli and Micrococci being the most common, sometimes occurring separately and sometimes in association, and then either alone or mixed with Torulæ or with Moulds (see Plates 1–3, Figs. 3, 4, 8, 9, 11, 16).

The Moulds met with have varied a good deal in kind. In some there has been only a mere commencing or incipient mycelium, as in Plate 2, Figs. 9, 10, 11; in others they have been more developed, and often mixed with conidia, as in Plate 5, Fig. 25. The bodies shown in Fig. 2, which I speak of as Fungus-germs, have been particularly abundant in many of the yellow solution tubes—and only in them. They are spherical, and often occurred in large masses, the heaps having then a brownish colour. They are evidently quite different from ordinary Torulæ.

In Plate 4, Fig. 20 A, there is shown a rudimentary Penicillium, and in Fig. 21 a more developed form of such a Mould—taken respectively from tubes Nos. 99 and 98.

Even a cursory examination of the Plates will suffice to show that we have here to do with no mere pseudo-organisms, such as have been described of late years by many workers with unheated solutions containing silica and various salts.[1]

Doubts have been previously expressed, however, as to whether the organisms taken from my tubes are really alive, and have actually developed therein.[2] This not unnatural scepticism has been partly due to the fact, as I have previously stated, that the Bacteria are always *motionless*, but probably to a far greater extent to a general disbelief in the possibility of "spontaneous generation."

Such difficulties are, however, easily met. It will be found that the organisms are generally most abundant and most varied in nature in the yellow solutions. But in them

[1] A full account of such investigations will be found in the works of Albert and Alexandre Mary, entitled *Etudes Experimentales sur la Génération Primitive*, 1909; and *Evolution and Transformisme*, 1910, Tome IV., pp. 305–325; as well as in a work by Leduc of Nantes, entitled *Theorie physico-chimique de la vie et générations spontanées*, 1910.

[2] See the report of the discussion which followed the reading of my communication to the Royal Medical and Chirurgical Society of London in the *Transactions* of that Society (Vol. XC., 1907, pp. 536–540).

there have been no solid ingredients introduced into the tubes—only freshly distilled water with a few drops each of dilute sodium silicate and of liquor ferri pernitratis. While in the colourless solutions the only solid ingredient has been a few grains of ammonium phosphate. After the heating process it is true there is a deposit of flakes of silica or of silicate of iron; but examination of portions of these after a few days will reveal no organisms, while an examination of the contents of other similar tubes after some months will probably show numerous motionless organisms (Torulæ or Bacteria, or both) on the flakes. But, as I have previously said: "If organisms are not there at first after the process of heating, and if after an interval they are there in abundance and are invariably stationary, clearly they must have developed in the sites where they are found."[1]

The growth and multiplication of the organisms, however, has often been followed in one or other of two ways.

(*a*) In some cases where it has been desired to preserve for a time a particular sample taken from a tube and found to contain organisms, the cover-glass has been at once surrounded with paraffin melting at about 105° F., in order to prevent evaporation of the fluid. Often in such cases, in the course of four or five days it has been found that a distinct multiplication of Torulæ or of Bacteria has taken place. Examples of this are shown in Plate 4, Fig. 23, and Plate 5, Fig. 27. Great multiplication of Torulæ had occurred, though the samples had been taken from tubes originally heated to 130° and 135° C. respectively.

(*b*) At other times, especially when doubts have been entertained as to whether what have been seen in the samples have been really Bacilli or Micrococci (Torulæ being, of course, always unmistakeable), an inoculation of another

[1] *Med. Chir. Trans.*, Vol. XC., 1907, p. 519.

sample from the tube has been made into a suitable thoroughly sterilised solution of a kind likely to favour their growth and multiplication.

The solution I have used for this purpose has been one containing ten grains of neutral ammonium tartrate and three of sodium phosphate to each ounce of distilled water. Such a solution, after it has been well boiled or superheated, favours the growth of such organisms, though it never engenders them. The mode of testing a sample is, therefore, quite simple. Four or five minims of the experimental solution with its deposit are dropped into a thoroughly sterilised two-ounce flask containing some of the recently reboiled test solution.[1] The flask is closed with a freshly boiled india-rubber stopper, and is then left in a warm chamber for from two to ten days or longer.

If Bacteria are plentiful, such a test solution will become more or less turbid in the course of from two to three days. Where Bacteria are less abundant, or less prone to multiply in the solution, the fluid often does not become turbid, but a sediment may distinctly increase in the course of from four to eight days. When a portion of this is removed by a pipette, it will be found to be composed of aggregates of Bacilli, or Bacilli and Cocci, mixed with Torulæ, such as are shown in Plates 1–5, Figs. 6, 7, 16, 22, 28. At other times, when there have been no Bacteria, only Moulds may be seen slowly developing at the bottom of the flask, and after a time rising to the surface and developing into distinct specimens of Penicillium with crowns of sporospores, as in Plates 3, 4, Figs. 19, 20; while, occasionally, where the organisms have been scarce, no result has followed from such inoculations.

[1] The flasks were sterilised by keeping them in a steriliser such as is used for surgical instruments, or else by putting them into an oven. In either case they were allowed to remain for over an hour, and the heat was sufficient to render cotton-wool slightly brown.

I have several times divided a recently inoculated test fluid into two portions, and have heated one of the portions to 100° C. for one minute, leaving the other half unheated, and have subsequently placed them side by side in the warm chamber. This I have done with samples taken from tubes that had been heated to 130° and also to 135° C., with the result that the fluid in the unheated test flask has speedily become turbid, or shown an increasing deposit, while the fluid in the other has always remained clear and revealed no trace of developing organisms—showing that 100° C. for one minute has been sufficient to kill them.

Again, where Moulds (Penicillium) have developed freely, in one of these test flasks, and have produced at the surface crowds of acrospores, I have well shaken the contents of such a flask and have then inoculated some minims of the solution into another sterilised flask containing some of the fresh test solution, and have boiled it for five minutes, and in another even only for one minute. I have two such flasks now before me, and after several months they still show no sign whatever of any developing Mould. Even one minute's exposure to a temperature of 100° C. has therefore been sufficient to kill the acrospores of these Moulds that have been developed in and from tubes previously heated to 130° or 135° C.[1]

In regard to the Plates, I may say further, that they begin with organisms taken from tubes that had been heated to 125° C., and go on to organisms taken from others that had been heated to 130° and 135° C.

I have also separated the illustrations of organisms taken

[1] This is entirely in accord with previous knowledge. De Barry, for instance, speaking of the death-point of the spores of Fungi in water or watery vapour, says: "It has not been shown that any can under these circumstances survive a temperature of 100° C." (*Fungi, Mycetozoa, and Bacteria*, Translation, 1887, p. 347.)

from tubes containing the yellow solution from those of organisms found in the colourless solutions, and it may be recognised that those from the former have, on the whole, been (while also more abundant) distinctly more varied in nature.

The organisms that were found in the experiments made in 1906 were in part similar to those now shown, but were in part different, as an examination of the Plates in my work *The Evolution of Life* will show. Some of the organisms found then, and shown in Figs. 8, 10, 30, seem not to have been previously known. Now also in Plate 2, Figs. 12, 13, something equally unfamiliar is represented, although such bodies have been very frequently met with in some of the yellow solutions.

EXPLANATION OF PLATES

PLATE 1.

Showing some of the Organisms obtained from Yellow Solutions that had been heated to 125° C. for 10″.

Figs. 1, 2, each × 500; Figs. 3–7 each × 700.

Fig. 1.—Group of ovoid Torulæ from tube No. 96.
„ 2.—Group of spherical Fungus-germs from tube No. 83.
„ 3.—Bacteria and Fungus-germs from tube No. 80.
„ 4.—Bacteria from tube No. 85.
„ 5.—*a*. Group of Torulæ, one developing a hypha.
b. Large Torulæ, with thick walls and granular contents.
c. Large Torula developing a hypha. All from tube No. 84.
„ 6.—*a*. Bacteria cultivated from tube No. 84.
b. Cocci and Bacteria cultivated from tube No. 83.
„ 7.—Torulæ and Bacteria cultivated from tube No. 80.

PLATE 2.

Other Organisms obtained from Yellow Solutions that had been heated to 125° C. for 10″.

Fig. 10 × 500; each of the others × 700.

Fig. 8.—A Zooglæal mass of Micrococci from tube No. 83.
„ 9.—Mass of Cocci and Bacilli, with developing Fungus-germs, from tube No. 80.
„ 10.—Portion of a small Mould from tube No. 97.
„ 11.—Bacteria and large Fungus-germs, also from tube No. 97.
„ 12.—A sheaf of delicate filaments, with a bubble of gas at the narrow end, in midst of granular deposit, from tube No. 85.
„ 13.—Portion of a much larger mass of the same kind, on which there are four bubbles of gas. Filaments can be distinguished through the small bubble, and around some of the others.

*** Such bodies have been common in some of the Yellow Solutions, and their constant association with gas-bubbles makes it probable that they are living organisms.

PLATE 3.

Some of the Organisms obtained from Colourless Solutions that had been heated to 130° C. for 10″.

Figs. 14, 15, 19, each × 500; Figs. 16–18, each × 700.

Fig. 14.—Shows the way in which very minute Torulæ are scattered over a flake of silica, from tube No. 104.
„ 15.—Crowds of much larger Torulæ from tube No. 24.
„ 16.—A group of Bacilli from tube No. 107.
„ 17.—Showing Torulæ from different tubes—Nos. 113, 54, 14.
a. A group showing germination.
b. and *c*. Torulæ of varying size.
„ 18.—Torulæ and Bacteria cultivated from tube No. 51.
„ 19.—A Mould *(Penicillium)* cultivated from tube No. 21.

PLATE 4.

Some of the Organisms obtained from Yellow Solutions that had been heated to 130° C. for 10″ (Figs. 20–22); also from a Colourless Solution heated to 130–133° C. for 10″ (Fig. 23); and from another heated to 135° C. for 5″ (Fig. 24).

Figs. 20, 21, 23, each × 500; Fig. 22 × 375, Fig. 24 × 700.

Fig. 20.—*a.* A rudimentary Mould *(Penicillium)* beginning to develop acro-spores, from tube No. 99.
b. and *c.* Torulæ from two different tubes—Nos. 61, 113.
„ 21.—Portion of a large mass of Mould, with heads of acro-spores *(Penicillium)*, from tube No. 98.
„ 22.—A *Penicillium* cultivated from tube No. 62.
„ 23.—Great development of Torulæ, from tube No. 113, found beneath cover-glass on fourth day after mounting.
„ 24.—Cultivation of Bacilli from tube No. 35.

PLATE 5.

Organisms from tubes that had been heated to 135° C. for 5″ (Figs. 25, 28 from Yellow Solutions; Figs. 26, 27 from Colourless Solutions).

Also Fig. 29, Organisms from an airless retort that had been heated to 100° C. for 20″.

Fig. 27 × 500; each of the others × 700.

Fig. 25.—Portion of a delicate Mycelium, with many spore-like bodies on the hyphæ, from tube No. 39.
„ 26.—Large budding Torulæ, some showing granules, and one a very distinct vacuole—all from tube No. 42, which, during the last five weeks, had been exposed to sunlight at a south window.
„ 27.—Multiplication of Torulæ, from tube No. 34, on under surface of cover-glass, as seen on fifth day after mounting.
„ 28.—Swarms of Torulæ and Micrococci cultivated from tube No. 45.
„ 29.—Organisms from an airless retort (No. 75) sealed during ebullition after 3″, and then heated in a can of boiling water for 17″.
a. Bacilli; *b.* and *c.* Torulæ showing vacuoles; *d.* Leptothrix-like filaments.

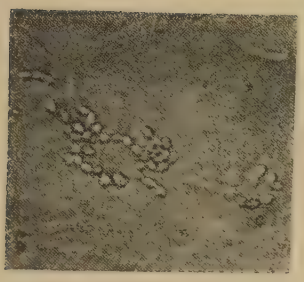


FIG. 1.

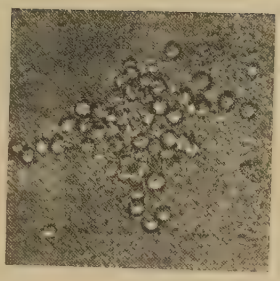


FIG. 2.

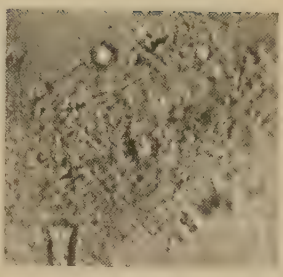
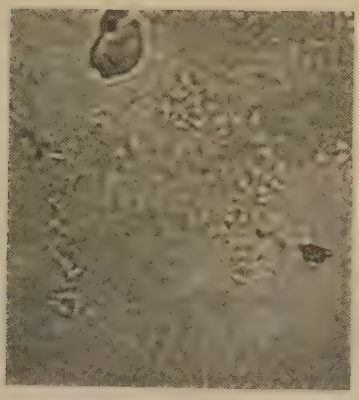


FIG. 3.



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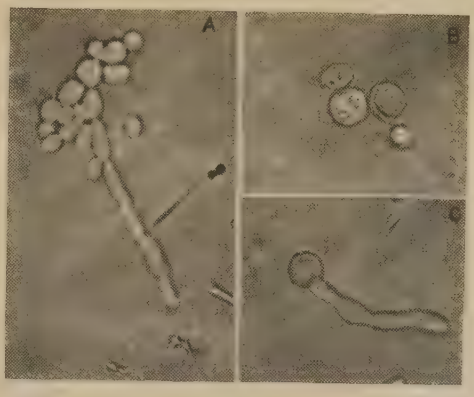
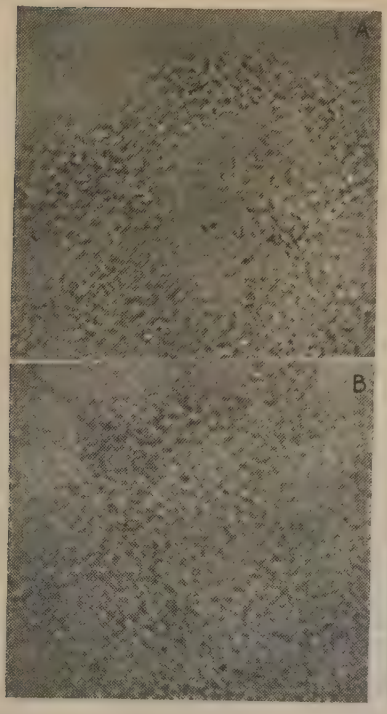


FIG. 5.



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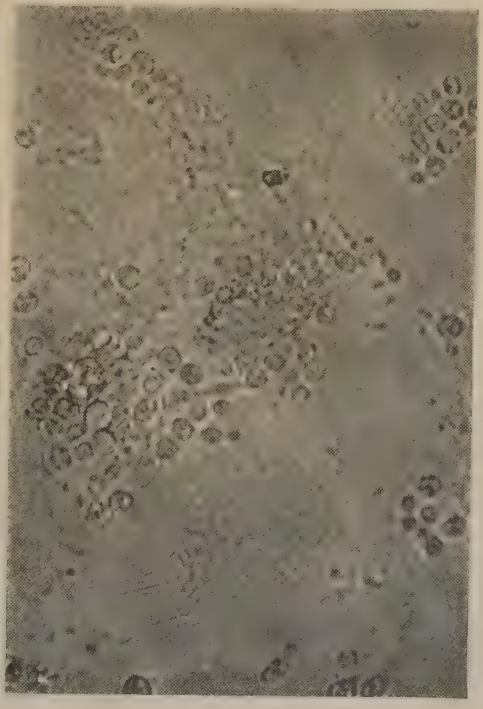


FIG. 7.

FIG. 8.

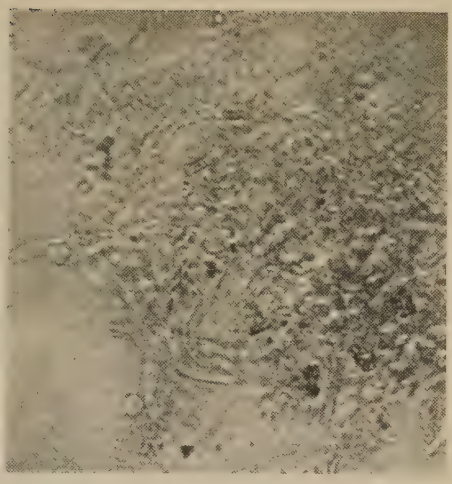


FIG. 9.

FIG. 10.

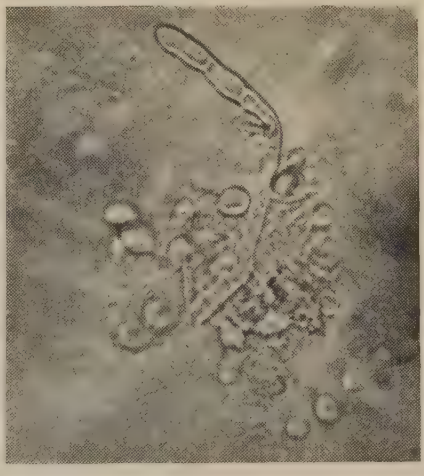


FIG. 11.

FIG. 12.

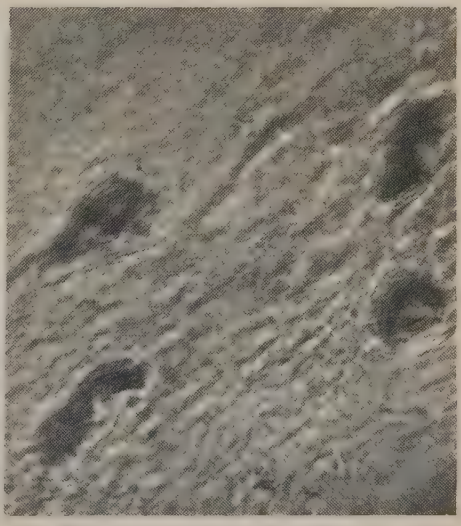
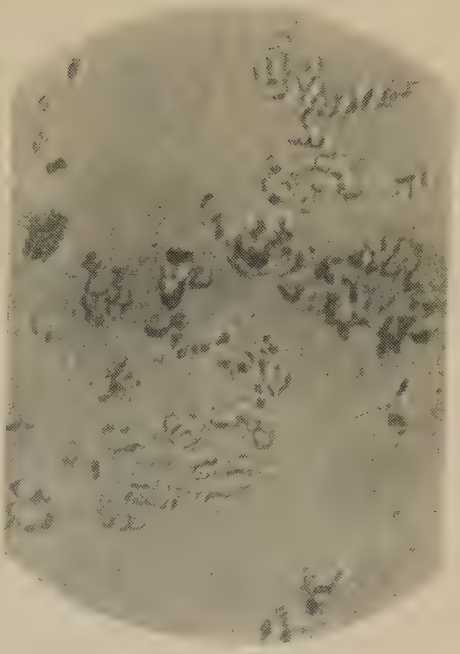


FIG. 13.

FIG. 14.



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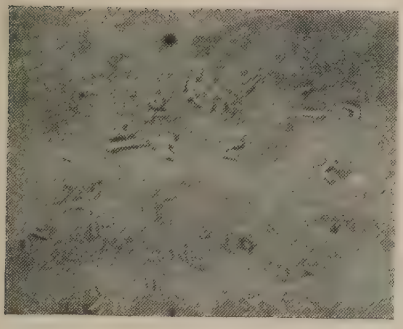

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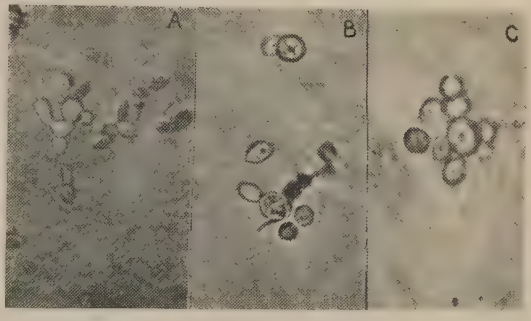

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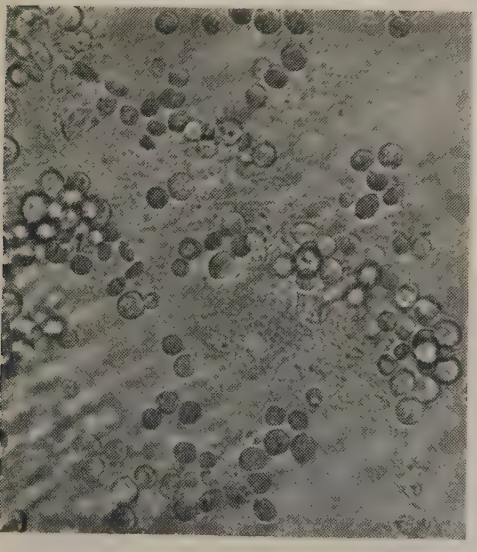

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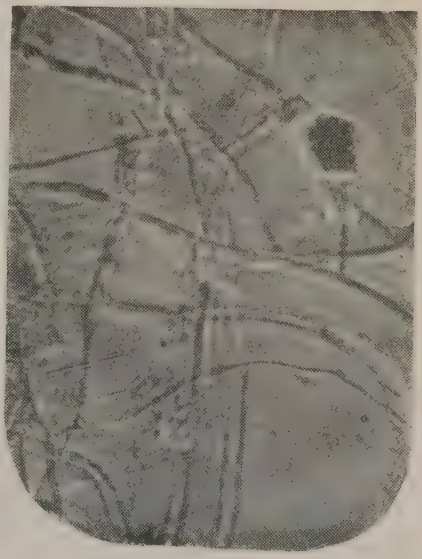
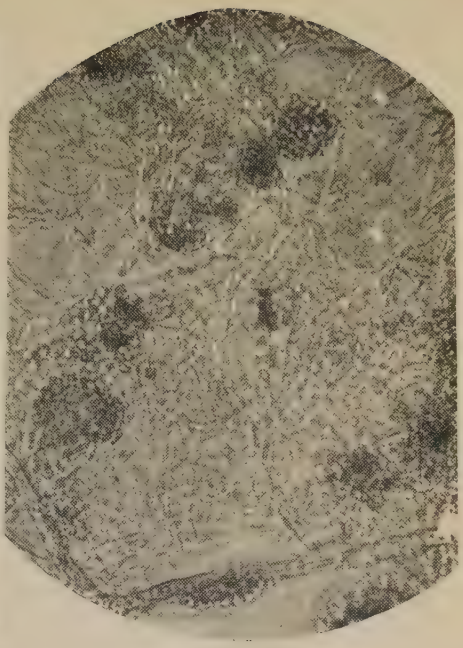


FIG. 19.

FIG. 20.



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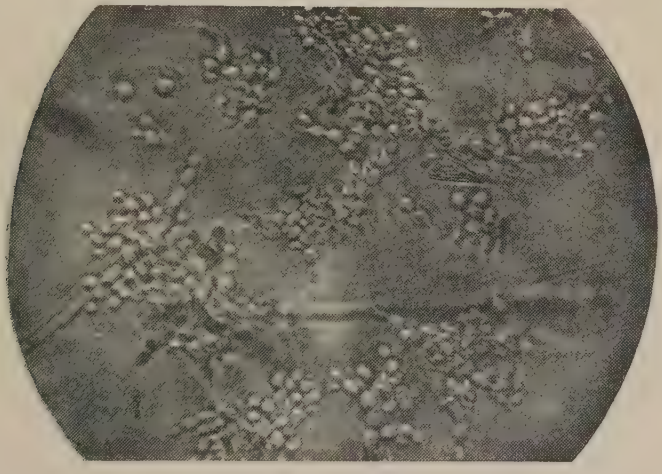

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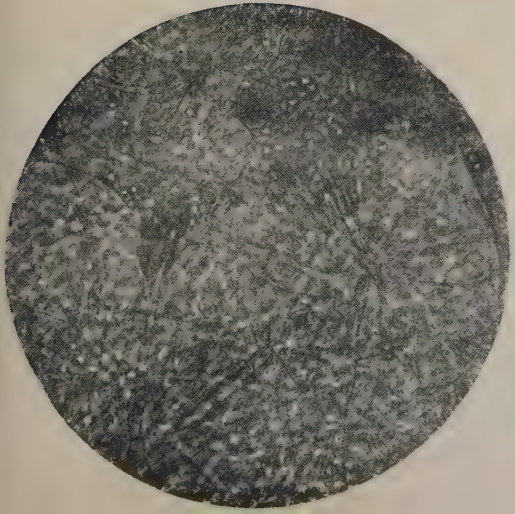

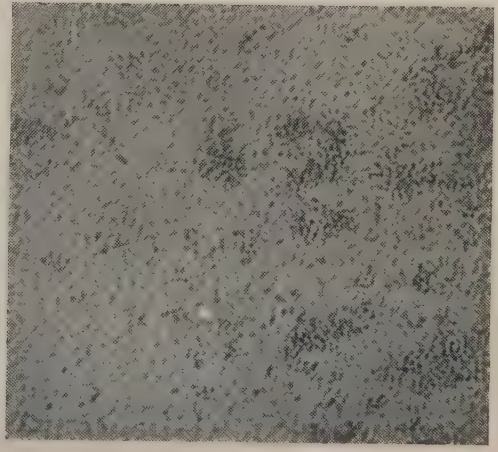

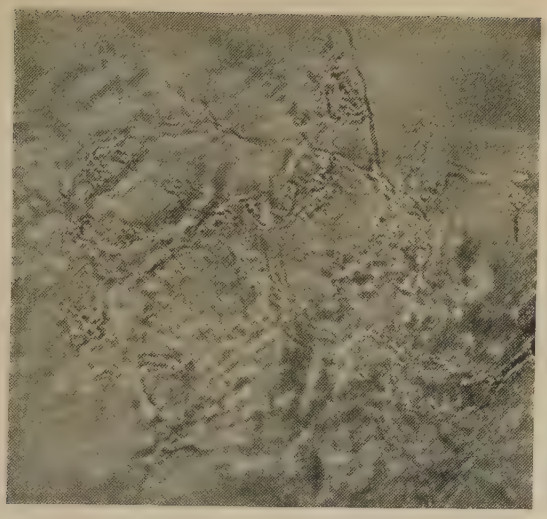

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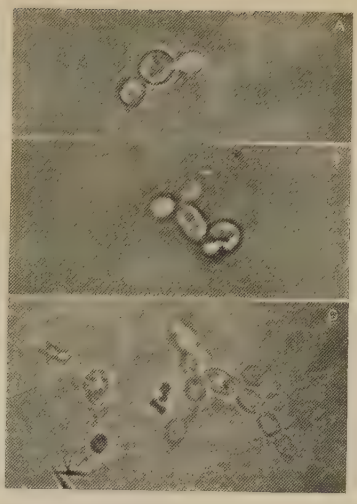
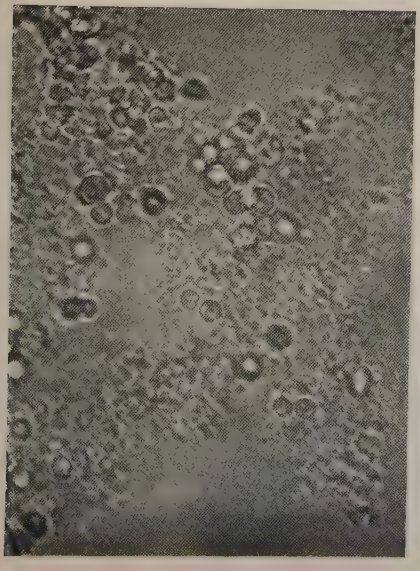
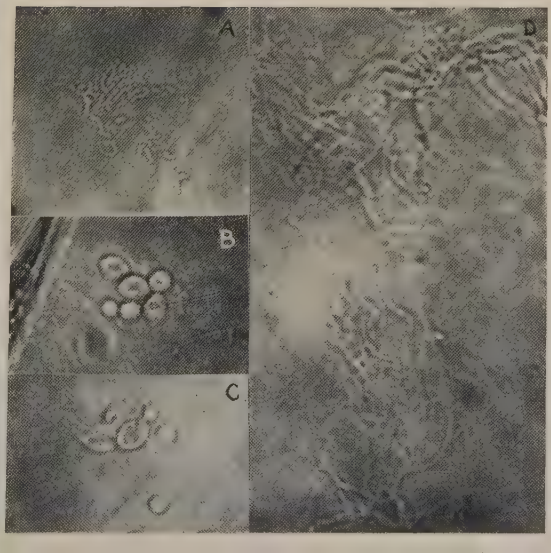


FIG. 26.

FIG. 27.





VI.—FURTHER EXPERIMENTS MADE IN 1910

THE numerous tentative experiments conducted during last March and April with different samples of sodium silicate, and the satisfactory results obtained with one of these samples, made me wish to perform further experiments with high temperatures and hermetically sealed tubes, making use of this particular sample,[1] and also employing it in diminished quantity, so that the solutions should yield only a very small amount of sediment. I hoped with it to obtain positive results in a much shorter time and in a still larger proportion of the experiments; as well as, by the modification indicated, to be able to find the organisms, when present, more easily in the scanty deposit.

Some time previously Professor R. T. Hewlett, of King's College, had written to me expressing interest in my experiments—"though a sceptic"; and, subsequently, I had several conversations with him in reference to them. When, therefore, I began to open some of my tubes, in March last, with positive results, specimens of the contents of a few of these were examined by him, and he became still further interested in the matter.

It was, therefore, finally agreed between us that I should make the contemplated further series of experiments in his laboratory, so that he might witness the preparation of the solutions, the opening and the re-sealing of the tubes, their subsequent heating, and ultimately be present at the examination of the contents of some of them, with a view to furnishing a short report as an addendum to this memoir.[2]

Accordingly, on April 28 I prepared 27 tubes under his

[1] That obtained from Messrs. Allen and Hanbury, of Vere Street, W.

[2] Unfortunately, owing to the serious illnesses of a member of his family, and other causes, Professor Hewlett has been unable to do the work originally contemplated, and supply a report on some of the experiments and on some of the organisms found in the tubes.

supervision, which were then heated in his autoclave, 20 of them to 130° C. for 10″, and 7 to 135° C. for 5″. Some days later 17 other tubes were also prepared in his laboratory, and were heated, with the aid of one of his assistants, to 130° C. for 10″. One of the tubes of this last series was accidentally broken, so that there were in all 43 new tubes, 23 containing the yellow solution and 20 the colourless solution.

In these experiments only two or three drops of the dilute sodium silicate were used to the ounce of distilled water. In the preparation of most of the yellow solutions I employed some of the supply of last year, as with it, in many of the tubes numbered 80–100, some of the most varied organisms had been found; while the new sample of sodium silicate was used for other of the yellow solutions, and for all the colourless solutions.

In preparing these latter yellow solutions, I at first used only two drops of the dilute silicate to the ounce; with the result that, after the heating process, the fluids were of the right pale port-wine colour, but with only the smallest trace of sediment. In the making of other solutions, therefore, with the intention of only slightly increasing the amount of deposit, I subsequently used three drops, instead of two, of this same dilute solution to the ounce; but, after the heating process, was disappointed to find that the solutions had all been decomposed, each tube showing a copious yellowish red, gelatinous precipitate, while the fluid above was perfectly clear and colourless.

In some of my tentative experiments I had previously met with this same kind of change, and, after a time, had found organisms, in some cases, in the deposit. The new tubes of this kind were, therefore, exposed with the others, in the hope that they might not be quite worthless.

All the tubes of this series have since been examined, and mostly in from six weeks to four months from the dates of

preparation; and in all, except in three of the above-mentioned decomposed solutions, organisms of various kinds have been found in more or less abundance.

Thus, out of the forty-three new experiments, positive results have been obtained in forty of them, though in seven of these latter (with colourless solutions) the tubes had been heated to 135° C. for 5″. In consequence of the very scanty deposit in some of the tubes, the centrifuge was used, and the last two minims of the solutions were then alone examined, with satisfactory results.

An examination of Plates 6–8 will, again, show that the organisms have been much more varied in nature in the yellow than in the colourless solutions. They have yielded especially various kinds of Mould, sometimes in comparatively large masses, and differing in kind from those that were found in the yellow solutions of the previous series. They yielded, however, none of the heaps of brown Fungus-germs, or aggregates of Micrococci, such as were so common in some of the tubes of the latter series, and are shown in Plate 1, Fig. 2, and Plate 2, Fig. 8. A moderate number of Bacteria have also been met with, and in the contents of three of these tubes I found, for the first time, a few motile Bacteria.[1]

Moulds have been distinctly less common in the colourless solutions. These have continued to yield Torulæ principally, either alone or in association with Bacteria.

In tube No. 136, for instance, both these kinds of organisms were found; and, in repetition of former trials with the contents of other solutions, I inoculated an ammonic tartrate solution with some of its deposit, then divided the

[1] In cases where Bacteria have been cultivated from the tubes in the ammonic tartrate solutions, they have frequently been very active; and Torulæ, too, when single and free, have commonly shown slight but distinct oscillations.

inoculated fluid into two portions, and heated one of the portions to 100° C. for one minute. The two flasks were then placed side by side in a warm chamber, with the result that the unheated portion became turbid with Bacteria and Torulæ in the course of three days, while that which had been heated remained clear—and so it continued after a lapse of two months.

Again, in tube No. 144, which had been heated to 135° C., my notes say that the Torulæ were "single or in pairs, but scarce." A specimen of the deposit to which this description refers was ringed with paraffin and put away in a drawer. When examined, after nine days, several beautiful groups of twenty to thirty Torulæ were found on the flakes of silica, where previously there had only been single specimens. Though the slip had been in the dark, the Torulæ had grown and multiplied remarkably. Perhaps the warming of the film by the hot paraffin had acted as an initial stimulus.

In Plate 8, Fig. 43, the largest mass of Torulæ I had, up to that time, ever taken from one of the previously sealed tubes is shown; while Fig. 44 represents a group of Torulæ, with distinct vacuoles, from another tube—one which had been heated to 135° C. Though the organisms are less varied in the colourless solutions, these fluids stand the high temperatures better than the yellow solutions; and the fact that they yield Torulæ so commonly supplies evidence especially convincing in regard to the main question—since it is universally admitted that a brief exposure to a temperature of 60–65° C. is fatal to all such organisms.

In these yellow solutions, as in those of the previous series, several bodies of uncertain nature have been met with. One of them was encountered over and over again in the form of what appeared to be mere irregular masses of granular material, though they were always associated with one or more minute bubbles of gas. Two of these bodies are shown

in Plate 8, Fig. 48; and the presence of the gas bubbles is certainly strongly suggestive that these aggregates may be organisms of some kind.

Being desirous of ascertaining whether positive results could be obtained with colourless solutions exposed to temperatures of 140° and 145° C., I had, on May 3, heated five tubes, charged with such a solution, to 141° C. for five minutes, and five others to 145° C. for five minutes. As the colourless solution used in the previous twenty experiments had contained only two drops of the dilute sodium silicate to the ounce, and still gave a larger amount of deposited silica than I desired, in these ten new experiments the solution was made with only one drop of the dilute sodium silicate to the ounce of distilled water.

After the tubes had been heated and cooled, I was disappointed to find that there was scarcely any deposit to be seen—only a very minute quantity in powdery form. All these tubes were at first put at the end of the north balcony, facing the east; but after about two and a half months they were placed just inside the south window, as I had by that time become disposed to believe they would then be under more favourable conditions. Inspection of the tubes at this time showed in each a slight though distinct increase in the amount of the deposit, which was of a light flocculent character.

Feeling assured that after exposure to these high temperatures the tubes ought not to be opened too soon, I allowed four months to elapse before beginning to examine any of the tubes heated to 141°, and just five months for those heated to 145° C. In almost all my previously recorded experiments I had been looking for organisms, and finding them, in and on the deposited silica. This light flocculent sediment was, however, quite unlike the flakes of silica found in the

previous experiments; and if my examinations had been limited to it the results with these tubes would have been comparatively unsatisfactory. It contained many strange and interesting things, in the form of concretions mixed with granules of different kinds and hyaline crumpled membranes, though only an organism here and there in some of the deposits.

But as in the case of some of the yellow solutions in which there was scarcely any deposit I had previously used the centrifuge, so now, after having removed as much as possible of the flocculent deposit with a sterilised pipette, I shook up the fluid rather briskly, so as to dislodge any organisms that might be adhering to the glass at the bottom of the tube.[1] The fluid was then poured into the tube of the centrifuge, and after its action all was removed with a pipette, except about the last two minims. The examination of them has in each case revealed Torulæ, or Torulæ and Bacteria in more or less abundance, with here and there an incipient mycelium.

All these tubes, but especially those that had been heated to 145° C., would have been better left for another month or longer, as the organisms were not abundant in any of them. In two of those that had been heated to this temperature (Nos. 164, 165), only a small number of single Torulæ (about twenty to thirty under each three-quarter-inch cover glass) were met with; while in another, in addition to the single Torulæ, two small groups, with Cocci (Fig. 51, A, B), were found. These three tubes had been at the south window for two months; though the remaining two, that had been heated to 145°, had only been there for one month. Yet in one of the latter the single Torulæ were much more abundant, and two groups, containing respectively ten and about fifty corpuscles, were also

[1] What was found in tube No. 178, and is recorded in the next section, made such a procedure seem desirable.

met with (Fig. 51, C); while in the other, though the Torulæ were scarce, there were many Fungus-germs different from any that had been previously met with (Fig. 53), though akin to those found in some of the yellow solutions, and which are shown under a lower magnification in Fig. 2.

This seemed to throw some doubt upon the question of the relative advantage of sunlight and mere diffuse daylight. Further observations will have to be made before a definite decision can be arrived at on this point. I have on several occasions, however, been much struck by the existence of marked differences in the number and even in the kind of organisms found in tubes containing portions of the same solution, similarly heated and similarly exposed. This was the case with tubes Nos. 162 and 163. In the former Torulæ and a few Bacteria were found; while in the latter, mixed with a few ordinary Torulæ, a number of Fungus-germs altogether different were found, such as had never previously been met with in colourless solutions. When some of them were first seen, I thought they must be dead, and due to some strange accidental contamination. To settle this point, the specimen under examination was at once carefully ringed with paraffin and put away in a drawer. When examined again on the third and fourth days, all doubts were at once removed, as many of the germs had undergone multiplication (Fig. 54); while many others were developing mycelia, a portion of one of which is shown in Fig. 55. It is certain, therefore, that these pale and brown Fungus-germs must, like the Torulæ, have been born in this tube, which had been exposed to the great initial heat of 145° C. for five minutes.

Then, again, the samples taken from tubes Nos. 161 and 162 were similarly ringed with paraffin; and by the fourth day Torulæ were found to have greatly multiplied, together with some Cocci, under each cover-glass. Some of these from No. 161 are shown in Fig. 52, A; while later, on the

ninth day, the hypha with lateral spores was seen which is represented in Fig. 52, B.

VII.—ADDITIONAL EXPERIMENTS MADE WITH PURE COLLOIDAL SILICA PREPARED BY GRAHAM'S METHOD

After mentioning, towards the end of last June, to Dr. Otto Rosenheim, the lecturer on Physiological Chemistry at King's College, the difficulties I had had to contend with owing to the varying composition of the sodium silicate of commerce, he kindly gave me a small quantity of pure, but dilute, colloidal silica. Graham says[1] this is "easily obtained in a state of purity, but it cannot be preserved. It may remain fluid for days or weeks in a sealed tube, but it is sure to gelatinise and become insoluble at last." When I wrote to Dr. Rosenheim in reference to this point, and to some of the results which I had obtained with his solution, his reply was as follows: "The solution is, as you assume, very dilute. It has been prepared according to Graham's directions, dialysed but not concentrated, and seems to keep indefinitely in this condition. I ought to add that I used a very pure sodium silicate and pure HCl for its preparation, and that all the salts have been dialysed away in a special dialyser with running distilled water."[2]

As soon as it was obtained I made one of the usual tentative experiments with it, making a guess at the amount which

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[1] *Phil. Trans.*, 1861, p. 183.

[2] Dr. Rosenheim has since informed me that what he gave me was some of a pure 0.01% solution of colloidal silica, the materials for which were procured from C. F. Kahlbaum, Chem. Fabrick, Berlin, whose agents here are Griffin and Sons, Kingsway, London. I shall ask the Berlin firm to prepare a 0.1% solution, of which one drop would be equivalent to the ten drops used by me for each fluid ounce of distilled water.

it would be desirable to use in the preparation of a colourless solution. I decided upon ten drops of this dilute solution to the ounce, using the other ingredients as before. This yielded a faintly acid solution, and after boiling it for ten minutes a minute amount of deposit was produced. The flask was closed with a sterilised rubber stopper, and placed on the north balcony. After fifteen days the flask was opened and the deposit examined, with the result that numerous small Torulæ single and in groups were found, together with some Micrococci.

This very speedy result surprised me, and I was at once anxious to try some further experiments with a high temperature and hermetically sealed vessels. I accordingly went to King's College; and, having none of the ordinary tubes at hand, four thick test tubes were drawn out charged with some of the same kind of solution as before, carefully sealed, and then heated in a bath of colza oil, with the intention of exposing them to 130° C. for 10″; but the temperature went up to 132°, and two minutes after it had reached that point two very loud explosions occurred. The heating was therefore stopped at once, and it was subsequently found that the bottoms of two of the test tubes had been blown off, though the other two were intact.[1]

One of these tubes was on June 28 placed on the north balcony, and the other at the south window, in order to get further evidence as to the relative advantage of the two modes of exposure. After only a little more than four weeks—that is, on August 1, both tubes were opened and their contents examined with the aid of the centrifuge, as there was only a very scanty deposit in each of them. In the tube which had been at the south window an extraordinary number of Torulæ

[1] Accidents of this kind seem never to occur with the soft soda glass. The harder glass of the test tubes is more like the uviol glass with which I had several such accidents in 1906.

were found in fragments of what appeared to have been a layer lining a part of the bottom of the tube (No. 178), a portion of which is shown in Plate 10, Fig. 58. In the tube from the north balcony the same kind of Torulæ were found, but in much smaller quantity. They were at least twenty times as numerous in the tube that had been at the south window.

Two days later I charged eighteen of the ordinary tubes with a solution in which there were twelve drops of the colloidal silica instead of ten to the ounce of distilled water. Six of these tubes were heated to 125°, six to 130°, and six to 135° C., each set for five minutes.

At first there was no appreciable sediment to be seen. All the tubes were placed at the south window, and when examined on the following afternoon, after they had been exposed to bright sunlight all the morning, a very slight flocculent deposit was observed in each, and not appreciably less or more in the tubes that had been heated to 125° than in those that had been exposed to 135° C.

All these tubes were opened at periods varying from four to eight weeks, and in every one of them organisms were found in more or less abundance—principally Torulæ and Bacteria, though a few small Moulds have also been met with.[1] In their examination I at first withdrew some of the light flocculent sediment with a sterilised pipette, and, when taken from the earlier tubes, without finding in such sediment a single organism; but in tubes opened after the fifth week Torulæ and also Cocci have sometimes been found in this situation in small quantities. In the examination of each

[1] I say "more or less abundance" because there was the same kind of variation here as that to which I have referred in the last section, in the abundance of organisms. Thus in tube No. 187, my notebook says: "Crowds of small ovoid Torulæ were found in small groups and singly"; while in No. 188, similarly exposed: "Only solitary Torulæ, comparatively scarce, and no groups were seen." There was a similar small quantity of organisms in tube No. 190.

tube, however, after this deposit had been scrutinised, the centrifuge was used ; and then, after almost the whole of the solution had been withdrawn from its tube, the contents of the last two minims were also carefully examined with the microscope. Torulæ, single, budding, and in connected groups such as are shown in Plate 10, Fig. 60, C, were found in varying quantity in different tubes, either alone or associated with Bacteria. In some of the tubes large masses of Bacteria were met with mixed with Torulæ (Plate 10, Fig. 59), while in others the Bacteria were in smaller numbers, forming scattered groups as seen in Fig. 56, showing organisms taken from a tube that had been heated to 135° C.

In each case the fluid in the tube was well shaken before the centrifuge was used, so as to dislodge the organisms from the bottom of the tube, where, with this solution, they seem principally to form, rather than in the light flocculent sediment.

In repetition of previous cultivation experiments some of the contents of tube No. 186 were inoculated into an ammonic tartrate solution ; and when this was examined, after nine days, though the bulk of the fluid was still quite clear, the flask contained a very distinct amount of sediment, which on examination was seen to be composed of masses of Torulæ intermixed with a few large Bacteria (see Plate 10, Fig. 61). A similar experiment made with some of the contents of tube No. 193, which had been previously heated to 135° C., also showed after nine days a very distinct sediment at the bottom of the flask, though this on examination was found to be principally composed of masses of rather large Bacteria intermixed here and there only with Torulæ (Plate 10, Fig. 57).

This last series of experiments with colloidal silica is an especially important one for three principal reasons. In the first place, a pure product has been employed rather than a

varying commercial article, so that others will be able, when making use of it, with more certainty to verify my results. Secondly, it has been shown that for this solution the tubes seem to be most advantageously exposed to actual sunlight inside a thick glass window, rather than outside to mere diffuse daylight. In this way they get the advantage of a warmer temperature as well as of a brighter light, the actual sunlight having most of its actinic rays cut off by the thick window-glass, plus that of the tube itself; so that, where not of too long duration, it seems to exert no harmful influence.[1]

While, thirdly, dealing with such a solution as I have employed in this last series of experiments, it seems that the combined effect of the higher temperature and the increased light leads to the appearance of organisms within the tubes in a comparatively short time, so that we can look forward with fair certainty to the appearance of living organisms within them in from four to eight weeks—the longer period being necessary if the preliminary heating has been as high as 135° C., and even then only a sparing number of single Torulæ, rather than groups, will probably be met with.

I have made no high temperature experiments with yellow solutions prepared with this colloidal silica, but expect that such solutions would yield equally satisfactory results, judging from certain tentative trials at 100° C., in which 12–18 drops of the colloidal solution were used, with eight drops of the pernitrate of iron, to the ounce of distilled water. In all the trials with this solution the boiled fluid became of a pale port-wine colour; and, with eighteen drops, a small but distinct

[1] A reference to the last communication of Sir Arthur Downes, entitled "The Action of Sunlight and of Diffused Light on Micro-organisms," *Proceed. of Royal Society*, 1886, will show that this is not as much as it may seem to be at variance with his experience. The amount of sunshine in the summer of 1910 was much less than usual. Perhaps had it not been for this, the exposure of tubes at the south window for one to two months might have been harmful. Further observations are needed.

amount of sediment was produced. After an exposure of three weeks at the south window, with the aid of the centrifuge, plenty of Torulæ and minute Moulds were obtained from the flasks.

VIII.—THE THERMAL DEATH-POINT OF SUCH ORGANISMS AS HAVE BEEN FOUND IN THE TUBES

An exposure for a minute or two in fluids to temperatures of 60°–70° C. is generally admitted to be destructive of Torulæ and all non-sporing forms of Bacteria.

All ordinary sporing forms of Bacilli are capable of resisting higher temperatures, owing to the greater resisting power of their spores. It is, however, generally admitted that these (*whether visible or so minute as to be invisible*) are killed in fluids when exposed for a few minutes to a temperature of 115° C.

There are certain extraordinary forms of Bacilli—the so-called "Thermophilic Bacteria," found in soils—whose spores are capable of resisting still higher temperatures. But, seeing that these have never been met with even in tap-water, and are still less likely to be found in distilled water, or in either of the chemicals that I have employed, they cannot be considered to be organisms with which we are at all concerned.[1]

That the statements above mentioned as to the heat-

[1] On this subject see *The Evolution of Life*, 1907, pp. 85, 282, or *The Medico-Chirurgical Transactions*, Vol. XC., p. 531. I may, however, state here that, according to Christen, they were always killed in 1–5 minutes at 135° C.; while W. P. Park found that 127° C. for two minutes sufficed to destroy them. Then, again, we have nothing to do here with the spores of Monads; but it seems well to mention that, according to Drs. Dallinger and Drysdale (*Monthly Microscop. Jrnl.*, Aug., 1873), the sporules of certain Monads are *sometimes* capable of surviving a *momentary* exposure in fluids to 268° F. (131° C.). Their trials, however, yielded very contradictory results, and have, I believe, never been confirmed by others.

resisting powers of such organisms as have been found within my experimental tubes are such as would generally be admitted may be gathered from the following quotation from a work by Jacques Duclaux, entitled *La Chimie de la Matière Vivante*, published early in 1910, and emanating from one of the workers in the Pasteur Institute. Speaking on this subject, the author says (p. 87) :—

> La résistance à la chaleur est, le plus souvent, bien moindre que la résistance au froid : les bactéries, dont un assez grand nombre supportent sans aucun dommage la température de −200°, périssent presque toutes dans l'eau bouillante, et il n'en est aucune qui supporte une température de 20° supérieure. Beaucoup plus sensibles, les levures de bières périssent entre 50° et 65°, et si on les échauffe graduellement, on constate qu'avant de mourir elles perdent graduellement aussi leur activité, c'est-à-dire leur pouvoir de provoquer des fermentations : car celles-ci deviennent très pénibles à 45° et s'arrêtent à peu près complètement à 50°.[1]

In regard to the tubes employed, it should be said, they have been sent to me hermetically sealed, and have only been opened for the purpose of charging them with their respective solutions, when they have been immediately re-sealed. The tubes themselves *have been thoroughly sterilised during the process of making*. Being little more than three inches long, in the process of rounding the lower end, and afterwards drawing out and closing the upper extremity, each tube has been made nearly red-hot throughout its whole length, as I have seen—having purposely been present during the making of some of them, in order to satisfy myself in regard to this point.

[1] On the question of the death-point of enzymes, the same writer says (p. 90): "Toutes les diastases ont comme la sucrase, une température optima et une température mortelle. Elles ne sont pas les mêmes pour toutes, mais il n'y a pas d'énormes différences, et, en général, la température de 75°–80° leur est rapidement funeste.....la sucrase, par exemple, provient de la levure, qui supporte difficilement 60°."

IX.—THE RESULTS OF THE EXPERIMENTS NOW RECORDED MUST BE CONSIDERED TO PROVE THE DE NOVO *ORIGIN OF LIVING MATTER*

As stated in Sections III. and IV., in the experiments initiated in 1909 living organisms were taken from fifty-two tubes that had been previously heated to temperatures of 125° and 130° C. for ten minutes, and from eight tubes that had been heated to 135° C. for five minutes.

In the first series of this year the results were as follows :— Living organisms were taken from thirty-three out of thirty-six tubes that had been heated to 130° C. for from five to ten minutes; from all of seven tubes heated to 135° C.; all of five heated to 141° C.; and all of another five that had been heated to 145° C. (293° F.) for five minutes; the three failures in the case of the tubes that had been heated to 130° C. only being due to the partial decomposition by heat of the particular yellow solutions employed in these experiments.

While in the second series of this year, in which pure colloidal silica was used, living organisms were taken from every one of the twenty tubes, which had been heated as follows:—Six to 125° for five minutes; six to 130° for five minutes; two to 132° for two minutes; and six to 135° C. for five minutes.

Thus, in the three series of experiments there have been twenty-one with positive results in which the tubes had been heated to 135° C. for five minutes; five in which the tubes had been heated to 141° C. for five minutes; and five in which they had been heated to 145° C. for a similar period.

The fact, therefore, that living organisms can be obtained, almost at will, from solutions which have been heated in hermetically-sealed vessels to temperatures very much higher than that which is known to be their thermal death-point

leaves no further room for doubt upon the much-contested point whether or not living matter is still capable of coming into existence. It now seems clear that what I have termed "Archebiosis" is a process likely to be constantly taking place in suitable situations over the whole surface of the earth, where conditions are favourable and not so restricted as those that have been necessary in our experiments.

That there is a limit to the amount of preliminary heating in these experiments, if living organisms are to be ultimately obtained, is probably due to the fact of the destructive influence of high temperatures upon the compound molecules of the colloidal ingredients of the fluids used. In the case of organic infusions this limit is much sooner reached than where we have to do with saline solutions, such as I have of late been employing. But it is now well known, as Graham showed, that the silica, obtained by dialysis from sodium silicate, exists in a colloidal state, in which it is represented by great compound molecules (micellæ) similar to, if simpler than, those of organic infusions.[1] It is doubtless due to this fact not only that solutions containing colloidal silica are capable of giving origin to what we know as living matter, but also that there are heat limits for them beyond which this process will no longer occur; these heat limits being, however, very distinctly higher than those for organic solutions—probably because the less complex molecules of the colloidal saline solutions are not so easily broken up by heat as are the micellæ of organic infusions. How these destructive heat limits are liable to vary for different

[1] Jacques Duclaux, in an article entitled "La Matière Organisée" (*Rev. Générale des Sciences*, Feb. 28, 1910, p. 139), says it has now become known that a solution of silica "contains micellæ formed of a great number of molecules of silica, combined together by the same sort of bond as that which unites the molecules of maltose into starch.....and this compound presents remarkable resemblances to natural organic matter." His use of Nägeli's term "micellæ" for the great compound molecules of colloidal solutions seems worthy of adoption.

combinations of the saline ingredients I have already briefly indicated (pp. 33–36).

It may well be asked, What possible explanation can be given of this process of Archebiosis occurring within the experimental vessels? And in answer one can only say that no explanation is as yet possible; nor can we even explain what is the nature of the force that binds this or that kind of molecule with others of like kind into such aggregates as exist in all colloidal compounds. Yet this, too, is a force very commonly in operation where it was previously little suspected, seeing that the molecules of various metals are now known to be capable of existing in colloidal combinations, as well as some of their compounds. Svedberg has published a list comprising about one hundred colloids of a mineral order.[1]

Still, in default of anything like an explanation, some few hints may be given tending to diminish the mystery attaching to the *de novo* origin of living matter.

In the first place, it would seem—so far as we know at present—to occur only in solutions containing colloidal compounds. And these, as Graham showed, are characterised by their mutability.[2] "Their existence," he said, "is a continued metastasis.....the colloidal is, in fact, a dynamical state of matter, the crystalloid being the statical condition. The colloid possesses energia. It may be looked upon as the probable primary source of the force appearing in the phenomena of vitality. To the gradual manner in which colloidal changes take place (for they always demand time as an element) may the characteristic protraction of chemico-organic changes also be referred."

[1] *Studien zur Lehre von den Kolloiden Lösungen* (Nova Acta Reg. Soc. Science, Upsala), (IV.), 2, 1907.

[2] *Phil. Trans.*, 1861, p. 183.

This, then, is the medium in which changes of a fermentative order seem to be brought about under the long-continued influence of suitable temperatures or that of sunlight. The changes are clearly of a fermentative order when we have to do with many organic infusions; though the phenomena are less obviously of this kind after such solutions have been subjected to very high temperatures, or when we have to do with saline colloidal solutions such as have been used in our experiments. In these latter cases organisms are met with *only in small quantities, mixed with some sedimentary matter.* These are not active, but slow and smouldering fermentations. In all such changes, as in most vital processes, destructive and constructive reactions are found to be associated. Concerning such constructive processes of a vital order, we have only to remember, as Professor Starling reminds us, that "every day, in all of us, the lifeless material of the food was being taken up and formed into the living constituents of our own bodies."[1]

But just as all fermentations are now regarded as immediately due to enzymes or chemical ferments (rather than to the "vital ferments" of Pasteur), so are almost all the elementary processes taking place in living things now being regarded as partaking of the nature of fermentations. In both, as just stated, there is the same kind of association of destructive and constructive chemical changes. It has been shown by G. Bertrand that even for the process of respiration a ferment (oxydase) is necessary, to seize the oxygen in the lungs, and hand it over to the red corpuscles of the blood. It thus seems legitimate to say, as Carl Snyder does in his *New Conceptions in Science* (1903, p. 234): "In brief, for every vital function a ferment. That is the latest word of biological chemistry. In broader terms, the sum of activities we collec-

[1] *Med.-Chir. Trans.*, Vol. XC., 1907, p. 536.

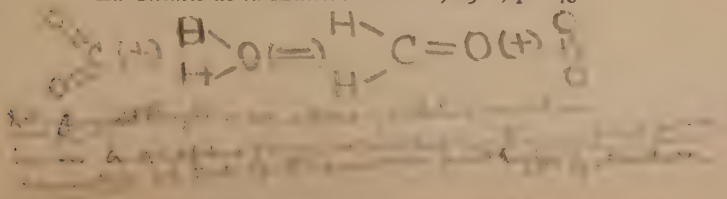
tively call cell-life is a series of fermentations." And he adds: "We may learn of the chemical synthesis of an enzyme any day, and that will be but the prelude to the manufacture of life in the laboratory."

In reference to the chemical side of this latter problem, it must be admitted that much light has been shed upon it during the last half-century. We know that protein compounds are the chief constituents of protoplasm, while the synthetic work of Emil Fischer, as well as investigations on the physiology of digestion, have shown that the proteins are, in turn, made up of compounds known as amino-acids, and that these are represented by varying multiple associations of carbohydrates, such as sugars, or formaldehyde (CH_2O)—the latter being the simplest term of the whole series.

But formaldehyde, which can be artificially produced by more than one method, is believed to be the first body formed in the nutrition of plants from CO_2 and water, under the combined influence of chlorophyll and sunlight; though its molecules are supposed to speedily combine, or polymerise, so as to form sugars such as saccharose and maltose. The molecules of these, in their turn, seem further to combine, so as to produce still more complex compounds—now appearing as the insoluble and savourless bodies known as starch (of which there are so many varieties), or else in the form of gums or cellulose. These bodies appear to be the most easily formed products in plants; and, though they seem all capable of being more or less easily transformed into one another, their synthesis has as yet baffled the efforts of chemists.

Still, the work of Fischer makes it possible that some day he or others may succeed and achieve the synthesis of one or other variety of albumen. Allied products have already been formed by him, as well as by Grimaux.[1] The chemist in such

[1] *La Chimie de la Matière Vivante*, 1910, p. 143.



researches has to advance step by step and by complicated processes, while in nature's laboratory, under the influence of those subtle catalysers known as enzymes, such bodies seem formed more or less directly.

How simply, by natural processes, even actual protoplasm can be engendered may be gathered from the fact that Bacteria and Torulæ, when introduced into the simple solution of ammonic tartrate and sodic phosphate in distilled water, will, under the synthetic influence of light, rapidly grow and multiply. What takes place in such a case may be regarded as a process akin to that which occurs in our saline solutions, or in organic infusions purified by heat, when fermentations, less or more marked in activity, are "spontaneously" initiated, with the result that multitudes of living units appear as the most notable synthetic products. We have, in each case, the formation of living matter out of the simplest elements, though the starting-point in the latter instances remains a mystery.

Still, we start in the experimental fluids with colloidal combinations which do not exist ready formed in the ammoniacal solution; and the cell substance or protoplasm of which the new-born units are formed is composed of varying combinations (of great complexity) of these colloidal molecules or micellæ. We can only suppose, therefore, that under the influence of heat, or of heat and light, these great molecules have been variously combined, though by stages wholly unknown, so as to initiate this or that kind of protoplasm. When we know what leads simple molecules to combine into the great colloidal molecules, we shall be in a better position for understanding how or why these micellæ again combine in this or that way, so as to form one or other of the innumerable varieties of protoplasm.

And in this relation it must be borne in mind that there is no abrupt demarcation between catalysers of mineral origin

and those formed in cells which go by the name of enzymes, the mode of action of the both being similar. This is well shown by Duclaux (*loc. cit.*, pp. 260–264). One of the most active enzymes—laccase—has been discovered by G. Bertrand to owe its oxidising properties mainly to the manganese which it contains; while other workers have found that manganese may be replaced by cerium, lanthane, or iron. So that, as Duclaux says, "this probably holds for all metals which yield soluble salts having two different degrees of oxidation that are easily transformable into one another."

Further, in reference to the initiation of living matter in our experimental solutions, there is also the fact, as stated by Duclaux (pp. 171–173), that under certain conditions silica itself is known to be a "particularly powerful" catalyser.

Once let the process be started in our experimental solutions by suitable catalysers, and then the *de novo* production of living units in the experimental vessels would be very closely comparable with the process above referred to (p. 64), in which we have to do *merely with growth and multiplication* taking place under the influence of catalysers contained within the bodies of the inoculating organisms.

X.—THE FORMS ASSUMED BY NEW-BORN UNITS OF LIVING MATTER

THE units of living matter that form in a colloidal solution, whether of organic or of mineral origin, must necessarily have their starting-point altogether beyond the range of ordinary aided vision. It is scarcely conceivable for an evolutionist to suppose that living matter could ever take origin, either now or in the past, in any other way. So that the birth of ultra-microscopic particles, gradually coming into the region of the visible, may easily be understood

to be a process lying altogether outside the range of man's experience; and therefore the establishment of the existence of such a process cannot, as of old, be truthfully said to "contradict the experience of all mankind."

Nor can the fact that the organisms found in the experimental vessels are common well-known forms, such as Bacteria and Torulæ, be with any logical force advanced against the doctrine of "spontaneous generation," though such a view was most strongly urged by no less an authority than Huxley, in opposition to my early experiments, when he said,[1] in reference to the finding of such organisms: "If these can be shown to be terms in the development of a known form, the probability of the same form turning up again spontaneously becomes by mathematical considerations infinitely minute; and for my part I could as soon believe that the calf I see grazing in a meadow had been spontaneously generated from the grass and flowers there." Language of this sort, from one who was naturally regarded as an authority on the subject, has doubtless helped for many years to stave off the recognition of a great truth.[2]

If, however, it is the fact that Bacteria and Torulæ are merely the primary forms most-frequently assumed by certain kinds of new-born living matter, then obviously the form and structure of these units, as well as of the Moulds into which the latter may develop, would stand in the same relation to the matter of which they are composed as the form and molecular structure of the crystal does to its matter.[3] There would be, in fact, just as much reason why the organic

[1] *Quart. Jrnl. of Micros. Science*, October, 1870, p. 361.

[2] Professor Huxley was at this time President of the British Association for the Advancement of Science, and the above utterance was made on September 13, 1870, during an address to a crowded meeting of the Biological Section "On the Relations of Penicillium, Torula, and Bacterium."

[3] Even though among the varied forms which appear, and are shown by the photographs, some of them actually take on the well-known form

new-born unit might develop into the likeness of one already in existence as there would be that the crystal of sodium sulphate which forms to-day in a solution of that substance should resemble that which formed under similar conditions hundreds of years ago, and which will similarly form a thousand years hence. He who believes in the uniformity of natural phenomena should anticipate no other result. Living matter which is now produced *de novo* speedily shapes itself into some well-known form ; and so, also, new crystalloid matter, which may have been produced synthetically by the chemist in his laboratory, falls naturally into one or other of the 230 known crystalline types. As G. H. Lewes aptly said,[1] "the link which unites all organisms is not always the common bond of heritage, but the *uniformity of organic laws acting under uniform conditions*."

We may, therefore, well recognise that the lower the forms of life—the nearer they are to their source—the greater is likely to have been the similarity among those that have been produced in different ages, just as the lowest forms are now practically similar in all regions of the earth. How otherwise, consistently with the doctrine of evolution, are we to account for the fact that different kinds of Bacilli and Micrococci have been found in animal and vegetal remains in the Triassic and Permian strata, in Carboniferous limestone, and even as low as the Upper Devonian strata?[2] Is it

of a Penicillium. See Plate 4, Figs. 20, 21, showing Penicillia taken from tubes that had been heated to 130° C., and others, as subsequently developed, from tubes similarly heated (Plates 3, 4, Figs. 19, 22). And yet, as previously stated (p. 41), the spores or conidia of such forms were all found to be killed when they had been heated for one minute to 100° C. Altogether different forms of Fungus-germs are, moreover, to be seen in Plate 9, Figs. 53, 54, 55, which proved to be living though they had been taken from a tube that had originally been heated to 145° C. for five minutes.

[1] *Fortnightly Review*, April, 1868 ; article on "Mr. Darwin's Hypotheses," p. 373.

[2] See *Ann. des Sciences Nat.* (Bot.), 1896, II., pp. 275–349.

conceivable that with mere lineal descent such variable living things could retain the same primitive forms through all these changing ages? Is it not far simpler and more probable to suppose, especially in the light of the experimental evidence now adduced, that instead of having to do with unbroken descent from ancestors through all these æons of time, as Darwin taught, and as is commonly believed,[1] we have to do, in the case of Bacteria and their allies, with successive new births of such organisms throughout these ages as primordial forms of life, compelled by their different but constantly recurring molecular constitutions to take such and such recurring forms and properties, just as would be the case with successive new births of different kinds of crystals?[2]

There is, in fact, now a strong consensus of opinion in the direction of views long ago expressed by Herbert Spencer when he said[3]: "As certainly as molecules of alum have a form of equilibrium, the octahedron, into which they fall when the temperature of their solvent allows them to aggregate, so certainly must organic molecules of each kind, no matter how complex, have a form of equilibrium in which, when they aggregate, their complex forces are balanced—a form far less rigid and definite, for the reason that they have far less definite polarities, are far more unstable, and have their tendencies more easily modified by environing conditions."

Weismann himself admits that if the power of repair and regeneration after injury, possessed to such a remarkable extent by so many of the lower animals, is dependent upon

[1] According to Darwin, "all the living forms of life are the *lineal* descendants of those that lived long before the Cambrian epoch."

[2] The 230 types of crystals are, as Prof. Liveing suggested, the outcome "of the accepted principles of mechanics"; and Prof. W. I. Pope said, in a recent discourse at the Royal Institution: "The aid of these, and these alone, has been invoked to show that crystalline structures result from the equilibrium of the attractive and repulsive forces radiating from atomic centres." (*Nature*, Aug. 11, 1910, p. 187.)

[3] *Principles of Biology*, 2nd ed., Vol. I., Append. D., p. 704.

a "primary power" rather than upon one that has been "acquired by Natural selection," he would have to give up his position, and therefore nearly all the hypotheses with which his name is associated. He says[1]: "In truth, if the body was really able to replace, after artificial injury, parts which were never liable to injury in natural conditions, and to do so in a most beautiful and appropriate manner, then there would be nothing for it but at least to regard the faculty of regeneration as a primary power of living creatures, and to think of the organism as like a crystal, which invariably completes itself if it be damaged in any part."

But, according to Loeb, Driesch, Oscar Hertwig, and many others, merotomy experiments, the powers of repair generally, and the fact of the development of one or three blastomeres into perfect organisms, all point to the faculty of regeneration being "a primary power of living creatures"; and the results of these investigations are regarded by them as being absolutely incompatible with the theories concerning biophores and determinants promulgated by Weismann.

Oscar Hertwig[2] holds with Nägeli that the idioplasm has a micellar structure, and that it is distributed equally to every product of cell-division; and such views are quite compatible with those urged by Herbert Spencer in opposition to the complicated theories and hypotheses of Weismann.[3] The forms of lower organisms are, in short, now by an increasing number of workers and thinkers regarded as in each case due to the necessary interactions between the particular kind of protoplasm (the isomeric varieties of which are absolutely innumerable) and the chemical and physical influences operative in its medium, tempered by the mutual interaction of the parts in their varying relations to the whole.

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[1] *The Evolution Theory*, 1904, Vol. II., p. 19.
[2] *Allgemine Biologie*, Dritte Auflage, 1908.
[3] *Principles of Biology*, Vol. I., 1898, Appendix B.

The *de novo* origin of a simple cell is now seen to be one of the earliest steps in morphology, although the actual stages of production are at present as inscrutable as are the marvellous "karyokinetic processes" we have of late years recognised as ultimately destined to take place in such bodies of a more developed order in which definite nuclei exist.

It is true that in Bacteria, and even in the Torulæ that are so constantly to be found in the experimental solutions, no definite nuclei are recognizable, still in these latter, under cultivation, vacuoles and even rudimentary nuclei show themselves (see Plate 1, Fig. 7).

While these are the most common forms of organisms met with in the solutions, other new or less familiar forms are encountered at times, as well as bodies concerning which it is difficult to say whether they are or are not living organisms.

XI.—THE SOLUTIONS EMPLOYED ARE NOT PRONE TO BE CONTAMINATED BY AIR-BORNE PARTICLES

Six tubes that had been heated to 130° C., in which no organisms had been found (four containing the colourless and two the yellow solution), were left in my study for six to eight weeks, standing upright, the opening in each tube being about one-third of an inch in diameter, so that dust particles could readily fall into the solutions.

The fluids, however, remained perfectly clear; and on microscopical examination of the deposit no organisms were still to be found after the periods mentioned.

This induced me to make other experiments bearing upon this question. I took four two-ounce sterilised flasks, and charged each of them with one ounce of the colourless fluid, which was then boiled in each of them for ten minutes.

Two of the flasks had a nicked rubber stopper, which, after the cessation of the ebullition, was pressed home. The other two flasks had no stopper, and the mouth of each was exactly half an inch in diameter, and was left freely open for dust to drop in; and yet, after the expiration of three months, the fluid in these two flasks (though diminished by evaporation) remained perfectly clear, like that in the two-stoppered flasks by the side of which they were standing.

A microscopical examination of some of the flakes of silica in each, moreover, showed just the same kind of Torulæ and Bacteria; and they were not more notably plentiful in that taken from the open than in that from the closed flasks, nor were any different organisms found in the former.

This trial was made with a solution of a kind which I had previously found to yield organisms readily (after it had been boiled for ten minutes) in the course of three or four weeks; so my conclusion was that in each of the four flasks organisms had been engendered *de novo*, and that multiplication had not taken place more freely in those that were open than in those that had remained closed. Again, after another period of three months, the fluid in each flask was found to be still quite clear and unclouded.

Yet I had previously found that when the same kind of solution was inoculated with *two drops of a solution swarming with Bacteria* from a fermenting fluid, either of animal or of vegetal origin, or with Martindale's three lactic Bacilli, or even with a pure culture of the *B. caucasicum*, the organisms multiplied in this solution, and that more or less distinct turbidity of the fluid showed itself after a few days.

Once, in another open vessel containing some of the same solution, a small patch of mould appeared upon the surface of the fluid, which, on examination, was found to have spread from a very minute dead fly; but even that has not occurred in either of the six open tubes or of the two open flasks. The

only conclusion to be drawn, therefore, is that the solutions which I have employed are not prone to be contaminated even by long exposure to air-borne particles.

XII.—DOES SILICON, EITHER WHOLLY OR IN PART, ENTER IN THE PLACE OF CARBON INTO THE COMPOSITION OF THE PROTOPLASM OF THE ORGANISMS FOUND IN THE TUBES?

THE question which stands at the head of this section cannot at present be answered. Still, it seems necessary to call attention to it for the following reasons.

It is now well known, as Professor Emerson Reynolds says,[1] that there are "a considerable number of silicon compounds, including nitrogen, which resemble those of carbon with nitrogen, both in composition and in the general nature of the changes in which they can take part. Some of these carbon analogues are closely related to those which are concerned in building up the organised structures of plants and animals.....In view of our newer knowledge, there is, therefore, nothing very far-fetched in supposing that, under suitable conditions, a plant or an animal organism may be able to construct from silicon compounds, ultimately derived from the soil, something akin to silicon protoplasm for use in its structure."

The fact of the existence of silicon alcohol and silicon ethers in which this element replaces carbon, together with the great similarity of the other compounds into which these two bodies enter, induced me to put forward this same supposition in 1872,[2] and to make some tentative experiments with different saline solutions "containing—in addition to nitrogen, oxygen, and hydrogen—some other element in the

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[1] *Nature*, August 12, 1909, p. 207.

[2] See *The Beginnings of Life*, Vol. II., Append. A, p. x.

place of carbon. The element with which the carbon was replaced was either silicon, boron, chromium, aluminium, or iron." And then there follows this statement: "Except in those in which carbon was replaced by silicon, no living things have been met with in any of these solutions (after they had been boiled and the necks of the flasks had been sealed during ebullition)." The solutions of this order in which organisms were then found were two only, and differed but very slightly from those with which I have been experimenting during the last four years. The ingredients were the same in each then as now, though their relative proportions differed slightly.

In the intervening period the same suggestion as to silicon being able to replace carbon in organic compounds has been made by others, including Clemence Royer, Alfonso Herrera of Mexico, Georges Renaudet, as well as Albert and Alexandre Mary.[1] It is, of course, well known to enter largely into the structure of the stems of grasses, bamboos, equisetums, and other plants.

Apart from these general considerations, there are the following special facts in reference to the foregoing experiments made with ingredients—namely, sodium silicate, ammonium phosphate, dilute phosphoric acid, and liquor ferri pernitratis—nominally containing no carbon, though CO_2 would be contained in the distilled water and in the air above the solutions, while carbon would also very probably exist as an impurity in the sodium silicate itself.

Still, if we bear in mind the following facts, it seems probable that there has been a partial substitution of silicon for carbon in the constitution of the organisms found:—

(*a*) The freedom with which moulds grow on the surface

[1] See the work of these latter writers entitled *Évolution & Transformisme* (Paris), Tome IV., 1910, pp. 306–315.

or in solutions of colloidal silica, as originally recorded by Slack and Chandler Roberts,[1] and the fact that green organisms, such as chlorococcus and its allies, when introduced into either of my early solutions, were found to grow and multiply, and that quite as freely in the colourless as in the yellow solution.[2]

(*b*) The fact that in my experiments the organisms have been invariably found away from contact with the air; never in the strata of water intervening between the deposited silica and the air; but always in and upon the deposited silica or at the bottom of the tubes.

(*c*) The fact that the same kinds of organisms have appeared, and also in similar situations, in experiments with the colourless solution in which the air has been expelled by boiling, before the tubes were hermetically sealed and subsequently further heated. (See Plate 5, Fig. 29.)

(*d*) Then, lastly, there is the fact that the solutions contain colloidal silica, and up to the present there is no good evidence that organisms can be engendered in any mere saline solution which does not contain colloidal silica.

Taken together, these facts are certainly very suggestive that silicon has replaced carbon, in part at least, in the formation of the protoplasm entering into the constitution of the organisms found within the experimental tubes. Such a supposition seems, indeed, as indicated in Section IX., to supply the only possible reason for the appearance of the organisms within the experimental tubes.

[1] *Quart. Jrnl. of Micros. Science*, 1868, pp. 105–108.

[2] The latter fact could not have been easily explained had it not been made known by Willstätter (*Liebig's Annalen*, 350, 48, 1906) that magnesium, and not iron, is the active principle of chlorophyll. Magnesium, I am told, would probably have been present owing to its having been dissolved out of the soft glass by the boiling solutions.

EXPLANATION OF PLATES.

PLATE 6.

Showing some of the Organisms obtained from Yellow Solutions that had been heated to 130° C. for 10″.

Fig. 33, × 300; Fig. 34, 500; each of the others × 700.

Fig. 30.—Two small Moulds with twisted hyphæ from tubes Nos. 153, 154.
„ 31.—Another small Mould with twisted hyphæ from tube No. 176.
„ 32.—A mass of Torulæ (with hyphæ from some of them) from tube No. 124.
„ 33.—A mass of Mould covered with spore-like spheres, also from tube No. 124.
„ 34.—Portion of a tangled mass of Mould from tube No. 129.
„ 35.—Another mass of a very similar Mould from tube No. 122.
„ 36.—Another Mould with large spherical sporangia from tube No. 129.

PLATE 7.

Showing other Organisms obtained from Yellow Solutions that had been heated to 130° C. for 10″.

Each of the figures × 700.

Fig. 37.—*a*. Large Bacteria from tube No. 130; *b*. A small much-twisted Mould from tube No. 176.
„ 38.—A Mycelium with much larger hyphæ, together with some Torulæ, from a colourless solution, No. 173, that had been heated to 130° C.
„ 39.—A tangled mass of Mould from tube No. 129.
„ 40.—A portion of a large mass of Mould with small spores from tube No. 176.
„ 41.—Portion of a large mass of brownish coloured Mould from tube No. 132.
„ 42.—A different kind of minute brown Mycelium, also from tube No. 132.

PLATE 8.

Showing Organisms obtained from Colourless Solutions that had been heated to 130° C. for 10″ (Figs. 43, 45–47), and 135° C. for 5″ (Figs. 44, 48, 49).

Fig. 49, × 375; each of the others × 700.

Fig. 43.—A large mass of Torulæ taken from tube No. 140.
„ 44.—A group of vacuolated Torulæ from tube No. 143.
„ 45.—Torulæ in the midst and on the surface of a feltwork of Bacteria from tube No. 173.
„ 46.—A portion of a mass of Streptothrix-like filaments from tube No. 136.
„ 47.—An aggregate of Bacteria on a flake of Silica from tube No. 149.
„ 48.—A group of very large Torulæ from tube No. 173.
„ 49.—Two granular-looking masses (presumably organic) with attached air bubbles from tubes Nos. 125 and 133.

PLATE 9.

Showing some of the Organisms obtained from Colourless Solutions that had been heated to 141° C. for 5″ (Figs. 49, 50) and 145° C. for 5″ (Figs. 51–55).

Fig. 55, × 375; each of the others × 700.

Fig. 49.—*a.* An incipient Mould (only a small portion not shown) from tube No. 159; *b.* Portion of a small Mould with unusually broad hyphæ from tube 157.

„ 50.—*a.* Groups of Torulæ and Cocci from tube No. 159; *b.* Two groups of Torulæ from tube No. 158; *c.* Mass of Cocci with two Torulæ (one out of focus) from tube No. 157.

„ 51.—*a.* Torulæ and Cocci from tube No. 161; *b.* Another group from the same tube; *c.* A large mass of small Torulæ from tube No. 162.

„ 52.—*a.* Torulæ and Cocci developed from tube 161, as found in mounted specimen on fourth day; *b.* Hypha with lateral spores which had developed in the same specimen by the ninth day.

„ 53.—*a.* Four large pale Fungus-germs by the side of a concretion; *b.* Six brown Fungus-germs; *c.* A large mass of similar brown Fungus-germs. All from tube No. 163.

„ 54.—Multiplication of Fungus-germs beneath cover glass of mounted specimen as seen on third day, from tube No. 163.

„ 55.—One of many Mycelia as found beneath the same cover glass on the fourth day.

PLATE 10.

Showing some of the Organisms obtained from Colourless Solutions prepared with Graham's pure Colloidal Silica that had been heated to 130° C. (Figs. 59, 61, 60 A, B); to 132° C. (Fig. 58); and 135° C. (Figs. 56, 57, 60 C.).

Each Fig. × 700.

Fig. 56.—Bacteria found in tube No. 193.

„ 57.—Bacteria cultivated from the same tube as found on the ninth day.

„ 58.—Portion of a large mass of Torulæ lining a portion of the bottom of tube No. 178.

„ 59.—Portion of a large mass of Bacteria and Torulæ taken from tube No. 189.

„ 60.—*a.* Portion of a Mycelium taken from tube 189; *b.* An incipient Mould from tube No. 186; *c.* A group of Torulæ, many of them united by minute hyphæ from tube No. 187.

„ 61.—A large mass of Torulæ, with four Bacteria, cultivated from tube No. 186, as found on the ninth day.

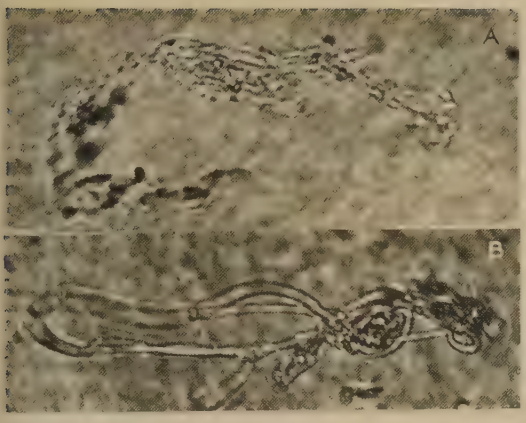
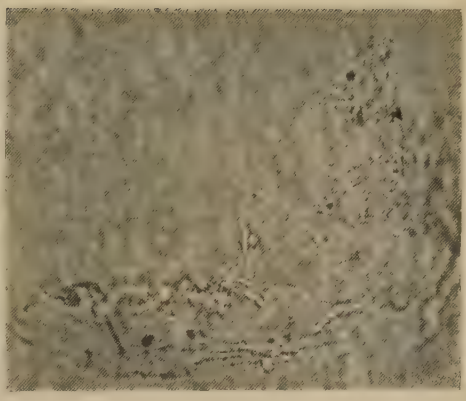


FIG. 30.



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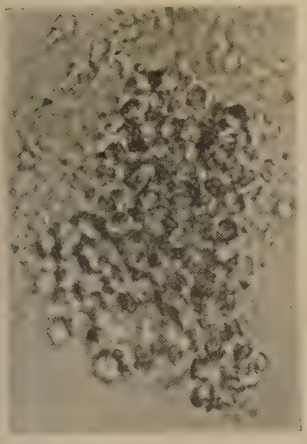


FIG. 32.

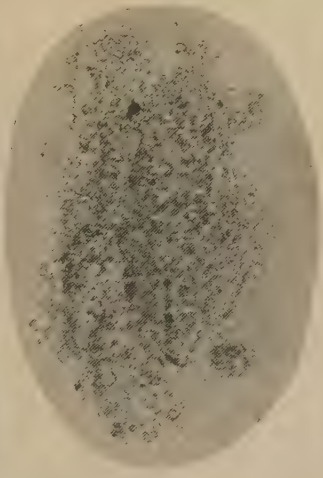


FIG. 33.

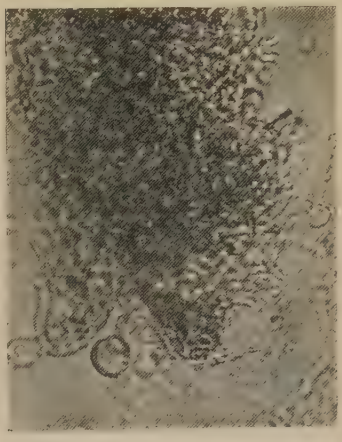
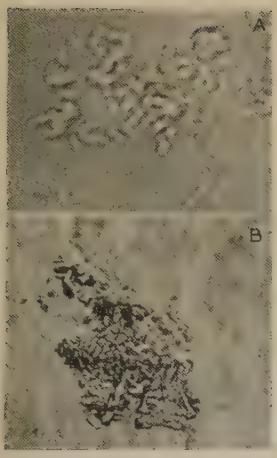


FIG. 34.

FIG. 35.

FIG. 36.



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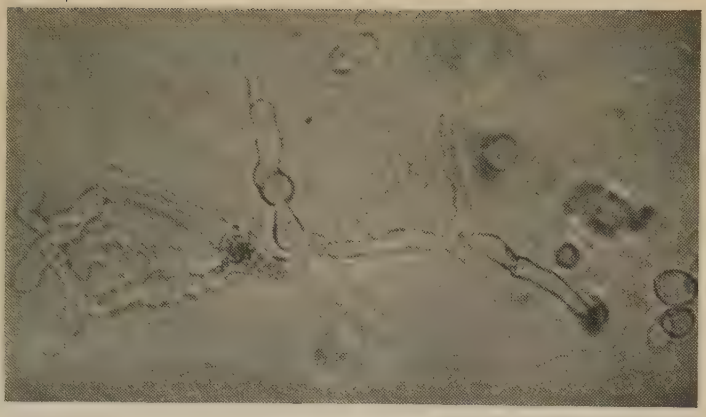

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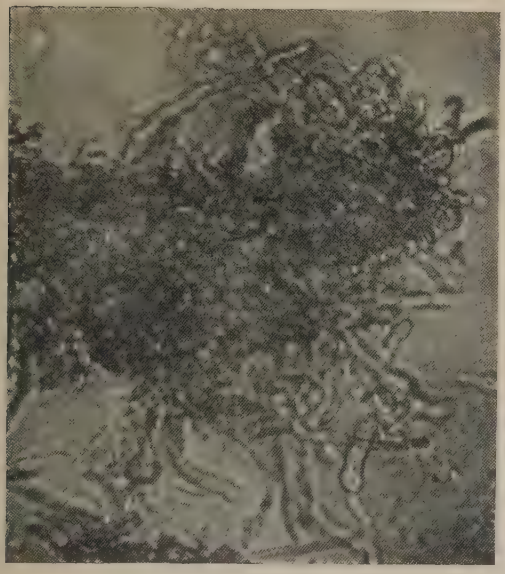

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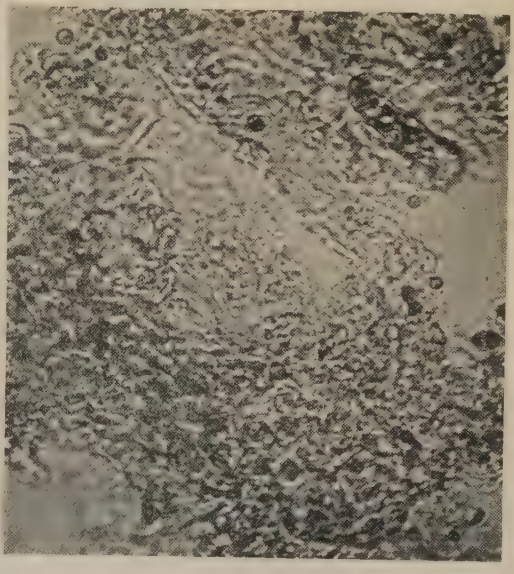

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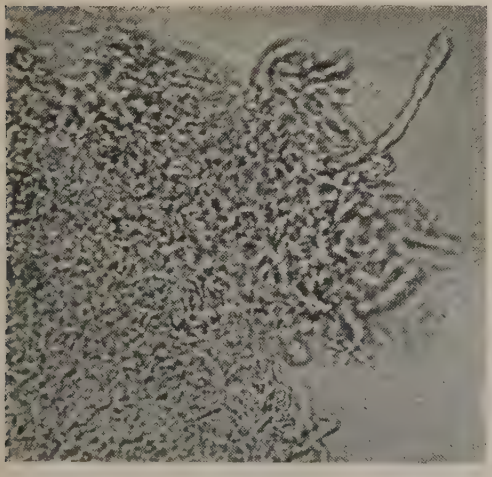

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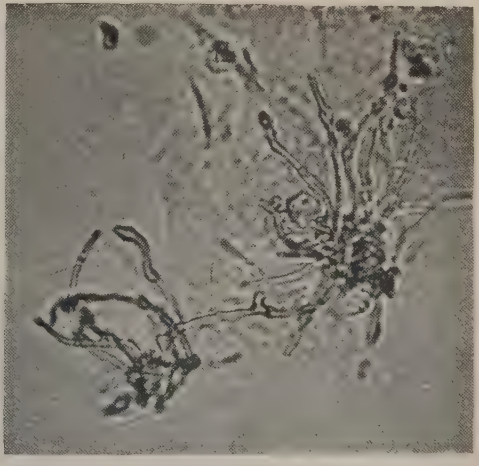

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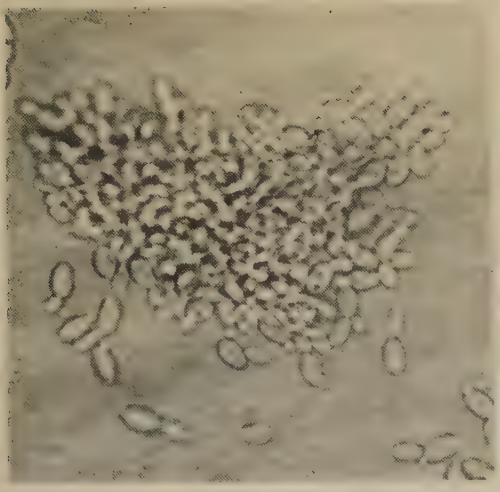

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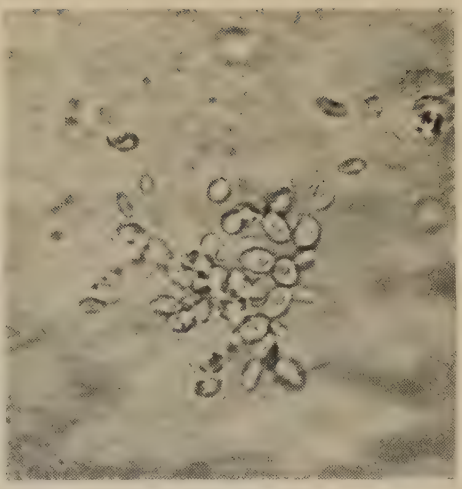

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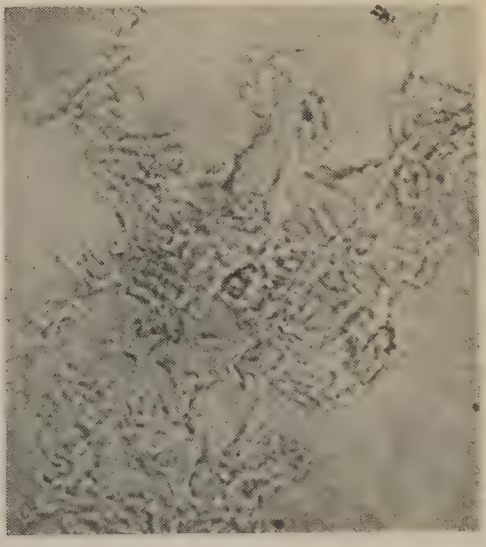

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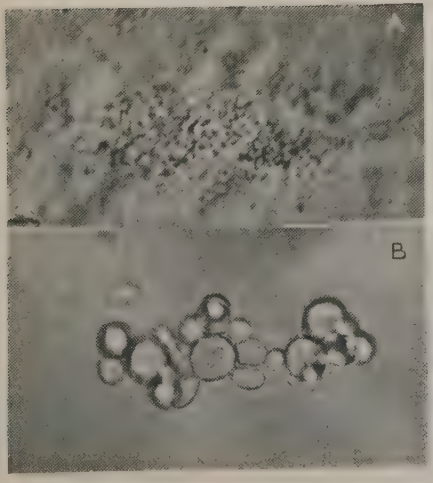

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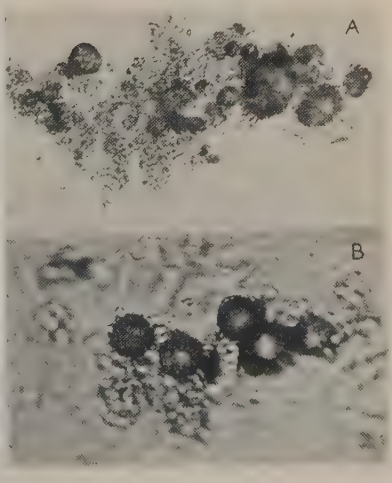

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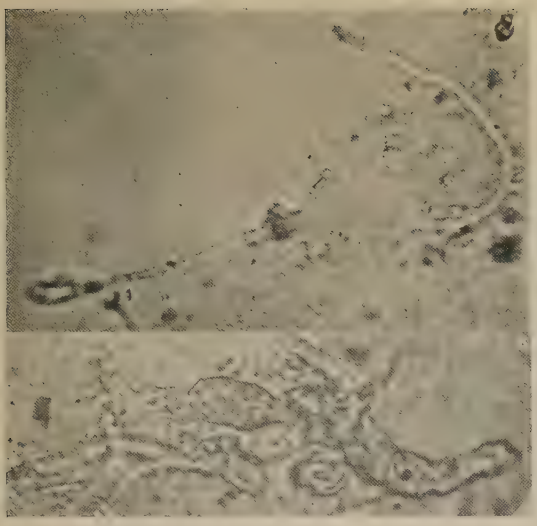

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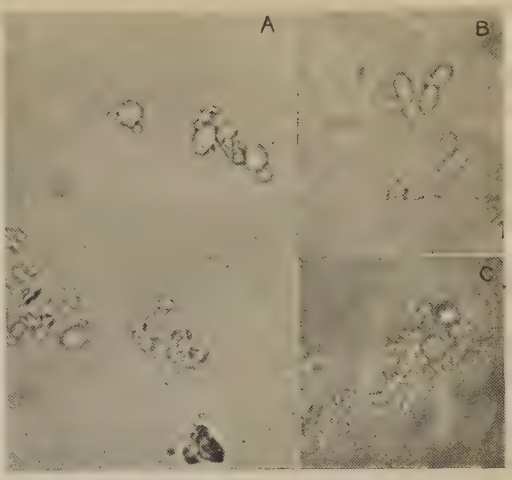

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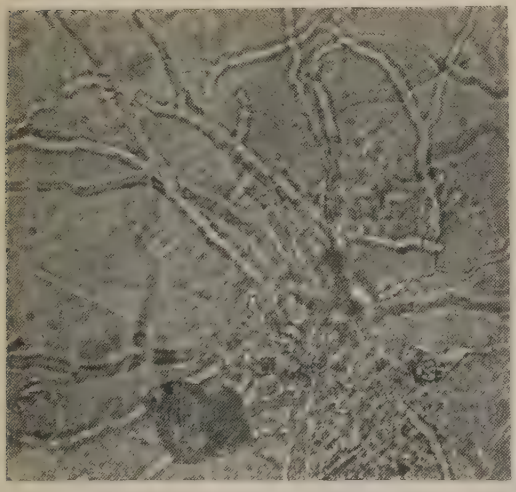

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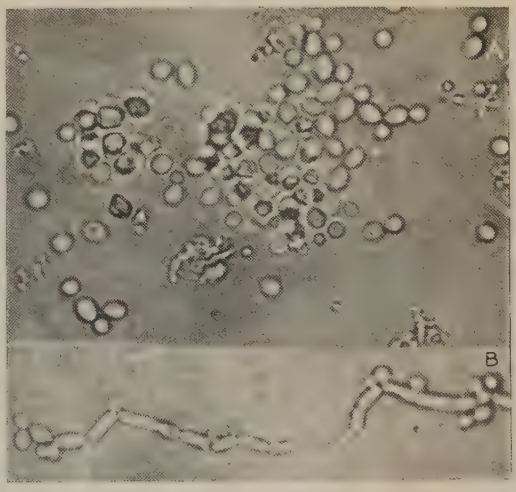

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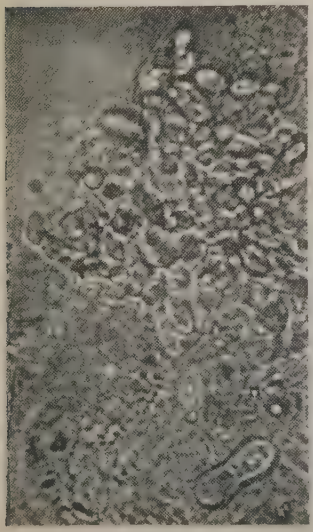

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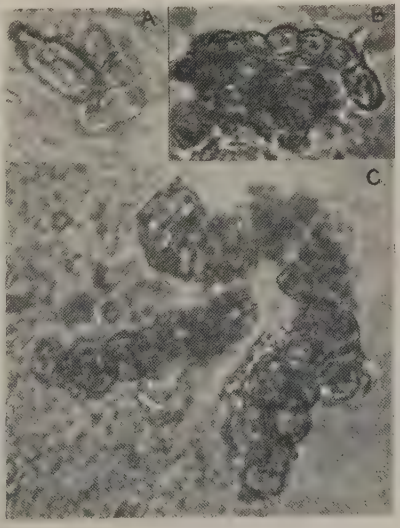

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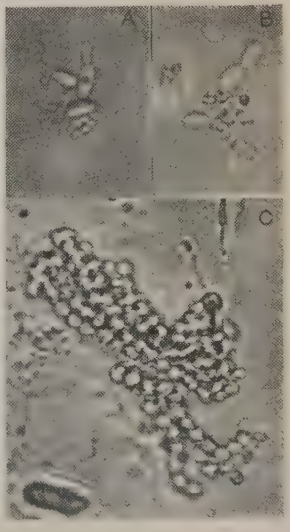
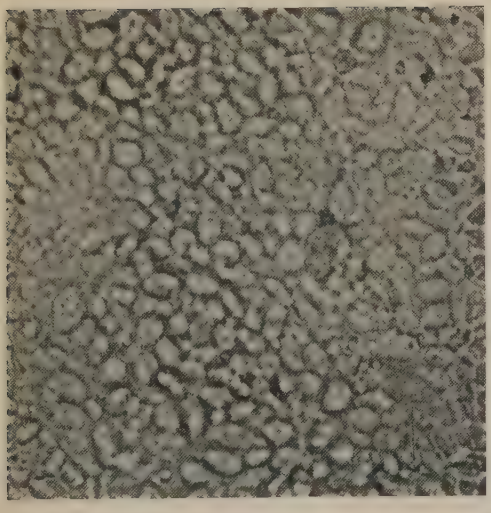


FIG. 51.

FIG. 56.

FIG. 57.



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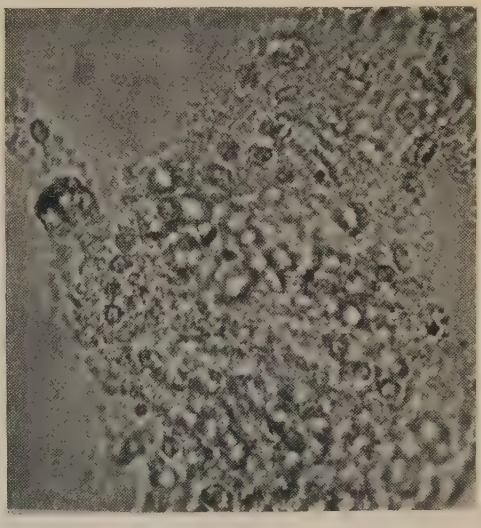

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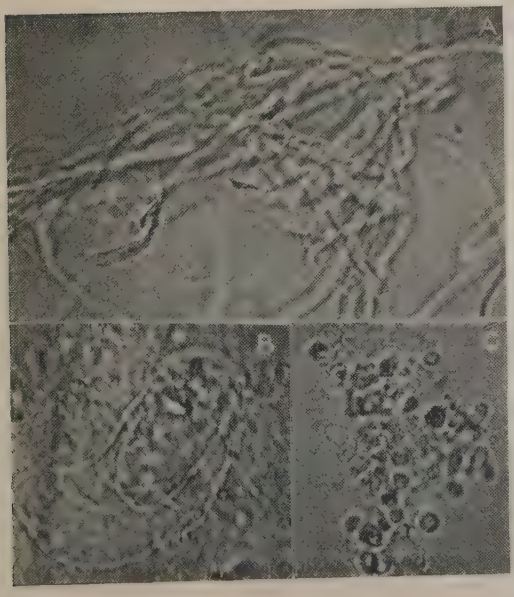

FIG. 60.

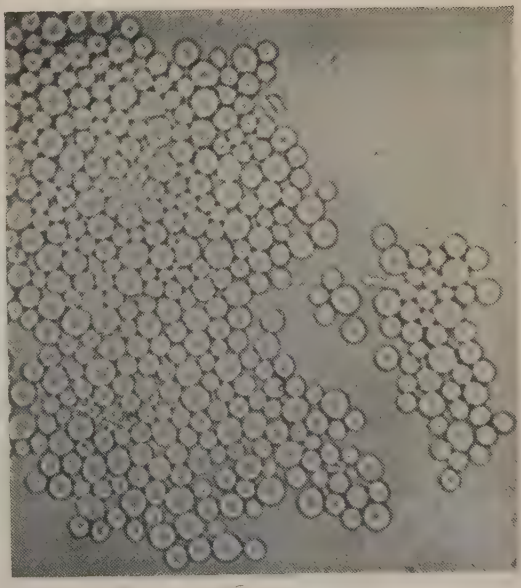

FIG. 61.

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